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ANALYTICAL STUDIES ON ACCOMMODATION OF MYOPIA

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Recently the increase of myopia has received much attention as one of the important social problems. Many investigators have attempted to find etyology of myopia, but it has not yet been proved thoroughly.

In these researches, there are some conspiquous reports on accommodative function in myopic eye. Ishihara and others suggested that accommodation of the eye had relation to age, but not so much to refraction, while Hönig first discovered that accommodation was influenced by refraction, and it was strong in hypermetropia and weak in myopia. Besides, Hagino, Yasui and others reported characteristic changes of accommodation with refraction.

In the present Study we attempted to survey accommodative function of myopia and the so-called pseudo-myopia from various angles in order to find the true cause of myopia and to improve its treatment.

Materials and Methods

104 male and female subjects between 10 and 25 years of age were chosen for the present study; they came to our clinic complaining disturbances of vision but in all of them no disorders were noticed except ametropias by clinical examinations. Examining the vision and refraction, we found that they were myopias having refraction errors from−1.0D. to−5.5D. skiascopically, with some myopic changes in the fundus of some of the subjects. Pseudomyopias complaining of troubles in vision were also found.

Light reaction time of the subjects was 175 milliseconds and sound reaction time of them was 160 milliseconds.

In the present study, to measure the accommodation of myopic eye the author used Hagino-Suzumura's Autographic Asthenopiameter (Fig. 1), which worked efficiently in analytical recording of accommodative functions for the criteria of syndromes. This instrument can represent eight kinds of pattern of accommodation, as seen in Fig. 2, but in the present study five patterns of them were selected in experiments.

Kinds of the test were as follows;

Test I: Measurement of accommodation time, i.e., contration time and relaxation time were measured alternately with five-second intervals (Fig. 2, No. 1).

Test II: Measurement of difference between contraction and relaxation times, i.e., these two times were recorded momentarily alternately (Fig. 2, No. 2).

Test III: Dotting method of appearance and disappearance thresholds of near point (Fig. 2, No. 5).

Fig. 1.

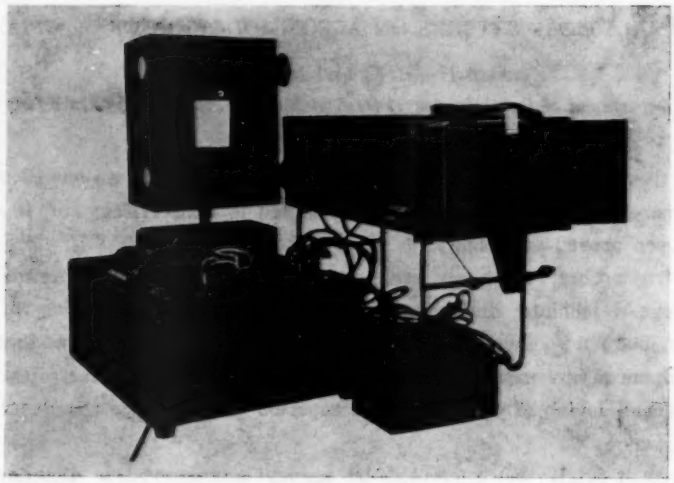
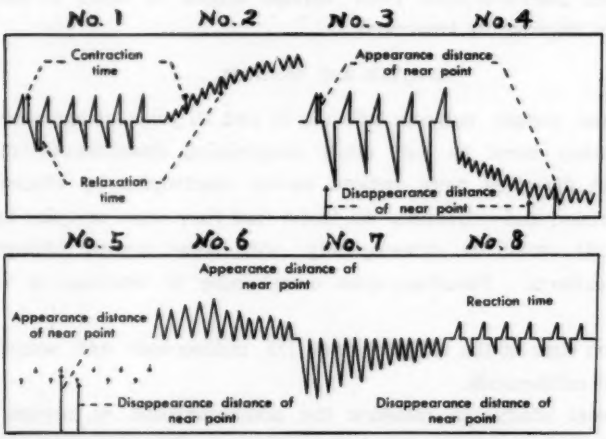


Fig. 2.



Test IV: Recording of distance difference between appearance and disappearance thresholds of near point, i.e., these two thresholds were recorded momentarily alternately (Fig. 2, No. 4).

Recording of reaction time: Light reaction time was measured by connecting the apparatus with an electronic counter (Fig. 2, No. 8).

Experimental conditions were as follows;

Experiment 1: Accommodative functions of each subject without any loading were measured.

Experiment 2: Accommodative functions of each subject were measured, corrected with the most adequate glasses, with which patients showed no complain for at least 30 minutes.

Experiment 3 : Measurement of accommodative functions after instillation of atropine was performed. For this experiment 1 per cent atropine was instilled twice a day for four days, and accommodative functions were measured after four weeks.

Results

Results are shown with some representative examples in the following lines with illustrations.

Experiment 1: Experiment 1 revealed that accommodative functions of each subject belonged to either one of the two types described below.

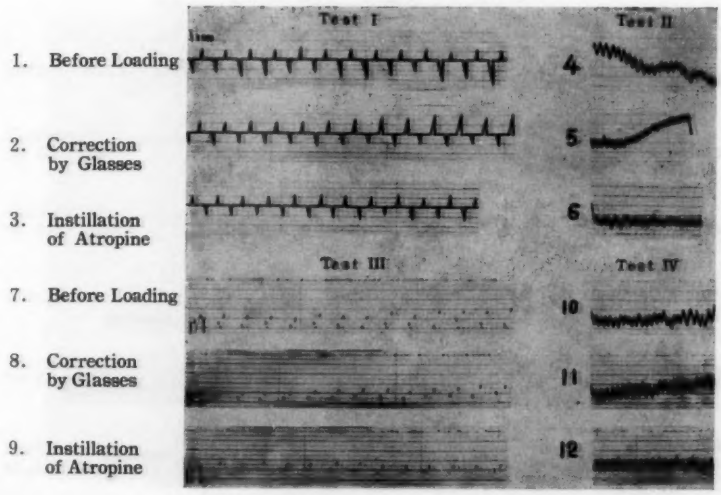
In Test I, as shown in Fig. 3-1, a slight prolongation of relaxation time, compared with contraction time, was seen at the beginning of measurements, and it gradually prolonged with repeated measurements.

Test II revealed that descending slope of the curve became greater, as relaxation time got longer with repeated measurements (Fig. 3-4).

Test III showed the tendency that the appearance thresholds of near point retreated gradually with repeated measurements (Fig. 3-7).

Test IV proved that repeated measurements gave little change in disappearance thresholds of near point, but made appearance thresholds retreat progressively, so that the difference between the appearance and disappearance thresholds of near point became larger as shown in Fig. 3-10.

Fig. 3. Type I.

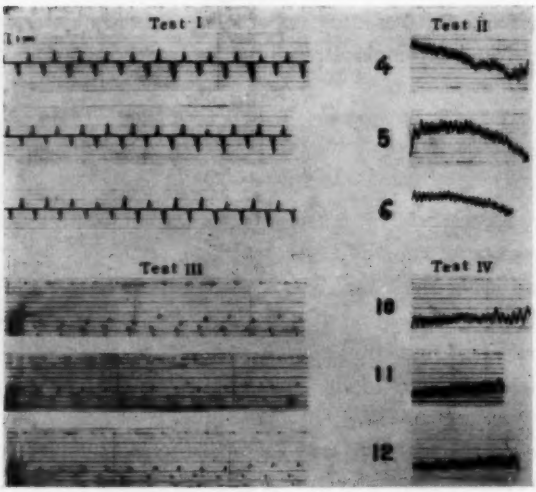


10 Year-old Girl

R.V.=0.7 (1.2×—0.5D) (Before loading.)

R.V.=1.2 (n.c.) (Instillation of atropine.)

Fig. 4. Type II.

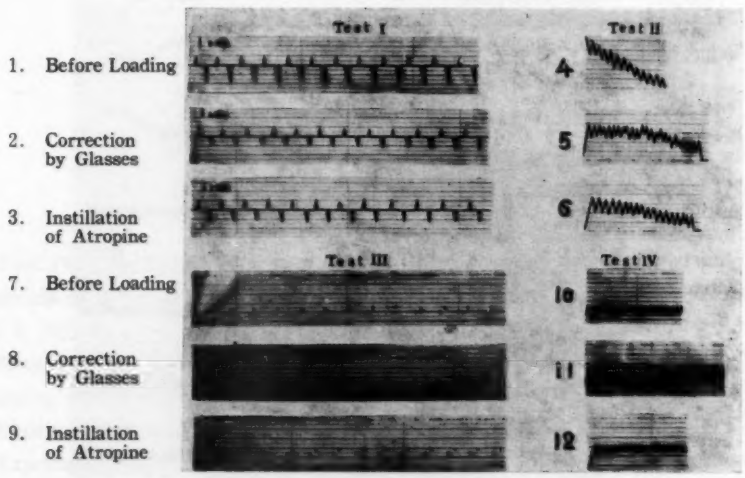


17 Year-old Boy

R.V.=0.7 (1.2×—0.75 D) (Before loading.)

R.V.=1.0 (1.2×—0.25 D) (Instillation of atropine.)

Fig. 5. Type III-(1)

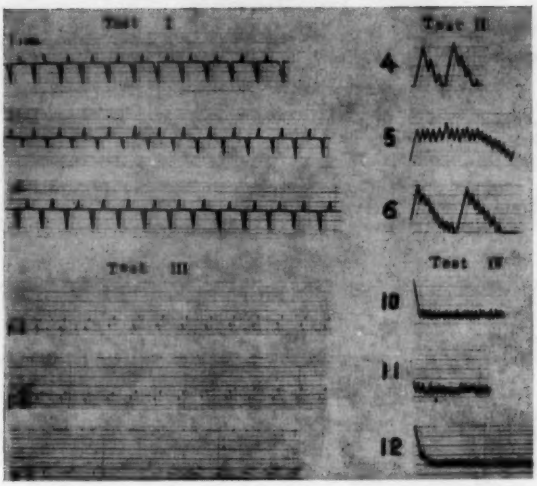


12 Year-old Boy

L.V.=0.4 (1.2×—1.25 D) (Before loading.)

L.V.=1.0 (1.2×—0.37 D) (Instillation of atropine.)

Fig. 6. Type III-(2)



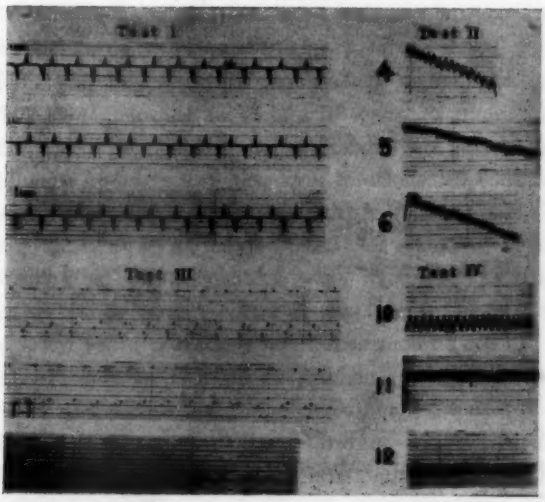
1. Before Loading
2. Correction by Glasses
3. Instillation of Atropine
7. Before Loading
8. Correction by Glasses
9. Instillation of Atropine

17 Year-old Girl

R.V.=0.2 (1.2×—2.0 D) (Before loading.)

R.V.=0.4 (1.2×—1.75D) (Instillation of atropine.)

Fig. 7. Type IV



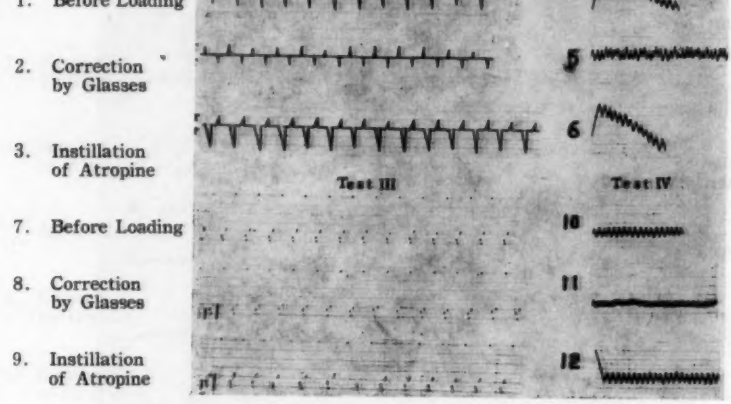
1. Before Loading
2. Correction by Glasses
3. Instillation of Atropine
7. Before Loading
8. Correction by Glasses
9. Instillation of Atropine

16 Year-old Boy

R.V.=0.4 (1.2×—1.5D) (Before loading.)

R.V.=0.4 (1.2×—1.5D) (Instillation of atropine.)

Fig. 8. Type V



18 Year-old Boy

R.V.=0.07 (1.2×—4.0 D) (Before loading.)

R.V.=0.07 (1.2×—4.0 D) (Instillation of atropine.)

Those cases described above in which relaxation time and appearance thresholds of rear point extended gradually with repeated measurements were classified as GROUP A.

In other cases, as shown in Fig. 7-1, 4, relaxation time prolonged remarkably in comparison with contraction time, and no change could be observed with repeated measurements in Tests I and II. Further, although appearance thresholds of near point retreated more than disappearance thresholds, any change could be found by repeated measurements of Tests III and IV as shown in Figs. 7-7, 10.

These cases having the tendencies of prolongation of relaxation time and retreat of the disappearance thresholds of near point were classified as GROUP B.

Experiment 2: Accommodative functions by correcting with glasses.

Accomodative functions of each subject in Experiment 1 were measured with the adoption of glasses. Through this experiment, all subjects could be devided into two types in *GROUP* A and three types in *GROUP* B as shown in Fig. 9.

Group A: In Test I contraction and relaxation times were almost the same at the beginning of repeated measurements, just as in emmetropia, but contraction time prolonged gradually with the repeated measurements, as shown in Fig. 3-2.

Test II showed wave pattern which inclined to ascend as a whole because of prolongation of contraction time from the middle of repeated measurements (Fig. 3-5).

Test III showed that both appearance and disappearance thresholds of near point retreated gradually and the retreat was less in the former than in the latter, although difference of these two thresholds were less at the beginning of repeated measurements

(Fig. 3-8).

Test IV revealed that the wave pattern inclined to ascend as a whole by the result of the retreat of both thresholds of appearance and disappearance in repeated measurements.

These cases as mentioned above were named Type I. On the other hand, mentioned in the following paragraphs were named Type II.

In test 1, in the beginning of repeated measurements, contraction time was unchanged but relaxation time showed slight shortening comparing with the time before loading in Experiment I, and so these two times were proved to be almost equal. But gradual prolongation of relaxation time was observed with repeated measurement (Fig. 4-2). Test II showed that the wave pattern inclined to descend as a whole, and it was converse to that of Type I (Fig. 4-5).

Tests III and IV showed no remarkable changes except a slight retreat of appearance thresholds of near point comparing with the case before loading (Figs. 4-8, 11).

All cases which were included in *GROUP* A could thus be divided into two types ···Type I and Type II.

GROUP B: In test 1, although representative myopic patterns were observed before loading, remarkable shortening of relaxation time was noticed by correcting with glasses, but it prolonged from the middle of repeated measurements showing almost the same change as Type II of *GROUP* A, as shown in Fig. 5-2.

Tests II, III and IV showed the same tendencies as Test I (Figs. 5-5, 8, 11).

Those cases explained above were designated as Type III. Considerable numbers in this type had the refraction of−1.25 D.

In cases shown in Fig. 7, Test I revealed that relaxation time was shortened by correction with glasses comparing with cases before loading, while contraction time unchanged, and that myopic change could still be seen since no fluctuations were observed with repeated measurements (Fig. 7-2). Tests II, III and IV also showed same tendencies as in Test I (Figs. 7-5, 8, 11).

These cases in which changes before loading became slighter by correction with glasses, but their tendencies of the pattern remained unchanged were designated as Type IV. Type IV could be seen in many cases which have refraction of between−1.50 and−3.0 D.

Fig. 8 was the illustration of the fifth cases: in Test I (Fig. 8-2), both the contraction and relaxation times were almost equal and no fluctuation was observed; Test II (Fig. 8-5) showed also no alterations of the two times, namely, since difference between the two times was very small, the wave pattern was parallel in form; in Tests III and IV, too difference between two thresholds of appearance and disappearance of near point became small and these two thresholds remained unchanged with repeated measurements (Figs. 8-8, 11).

As mentioned above, these cases showed the same change with emmetropia by

correction with glasses and they were called Type V. Many instances included in this type had myopic change in their fundus.

Experiment 3: Accommodative Functions after instillation with atropine.

All patients who were classified in the former experiments were subjected to measure their accommodative functions after four weeks since the time of instillation with one per cent atropine.

Type I: Test I and Test II showed no change in both contraction and relaxation times and these patterns revealed almost the same patterns as in emmetropia (Figs. 3-3, 6).

Tests III and IV showed the advancement of appearance threshold of near point and no retreat was observed with repeated measurements (Figs. 3-9, 12).

Type II: In Tests I and II, although contraction time showed no difference with cases before loading as in Type I, relaxation time was shortened, but it prolonged gradually with repeated measurements (Figs. 4-3, 6).

Tests III and IV revealed that disappearance threshold of near point retreated less than before loading but retreat was observed by repeated measurements (Figs. 4-9, 12).

Type III: This type had the similar change to Type II by the correction with glasses. Each case in this type, however, was divided into two subtypes by the results of the present experiment. One of them, as shown in Figs. 5-3, 6, showed good healing tendency like Type II with the instillation of atropine, and gave similar results to Type II with every test. The vision of these cases came to be 1.0 (1.2×−0.37 D.) by the treatment of atropine though it was 0.4 (1.2×−1.25 D.) before loading. This subtype of Type III was called Type III-1.

The other subtype was a group which had slight healing tendency by atropine instillation (Figs. 6-3, 6, 9, 12). In this group 0.2 (1.2×−2.0 D.) of vision was merely recovered to 0.4 (1.2×−1.75 D.) by instillation of atropine. Rather poorer healing tendency was observed, compared with Type III-1, although accommodative function by correcting with glasses showed almost the same with that type. They are designated as Type III-2.

Type IV: As shown in Figs. 7-3, 6, Tests I and II revealed that contraction time prolonged slightly but relaxation time was unchanged by instillation of atropine. From these facts, instillation of atropine to the subjects in type IV showed poorer efficiency.

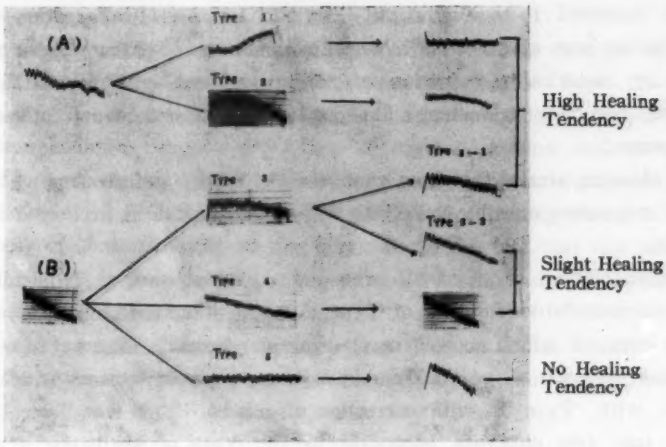
Type V: The ceses belonging to this type showed no changes by instillation of atropine in every measurement through all tests as shown in Figs. 8-3, 6,9, 12.

To sum up the results of these experiments, accommodative functions of myopias and the so-called pseudomyopias could be classified as shown in Fig. 9.

Discussion

Studies on the ethiology of myopia from the view point of accommodation are com-

Fig. 9. Correction by Glasses Instillation of Atropine



paratively few.

In the present study, the problems were discussed by using Hagino-Suzumura's Autographic Asthenopiameter for estimating the accommodative function analytically, with the interesting results as above mentioned obtained.

From the results concerning the cases before loading all subjected patients could be classified into two categories—Group A and Group B. Discussion were made on the concept of these two groups.

As to accommodative functions in Group A, no remarkable change was observed in contraction time, but relaxation time was prolonged with repeated measurements, with strong resemblance with initial myopia, designated by Hagino and Suzumura,or myopia in slight degree, by Teramoto. Changes in Group B were in good agreement with the case reported by Hagino, Suzumura and Teramoto. Thus, in all subjects, though states of change differed, prolongation of relaxation time was observed, with contration time unchanged; namely, in wave patterns, it is supposed that these changes show characteristically decline of relaxing function. However, when correction with glasses was applied, Type I showed prolongation of contraction time and retreat of disappearance threshold of near point by repeated measurements, but entirely no fluctuation was observed in relaxation time, contrary to the cases before loading.

Volkmer reported that the prolongation of contraction time was observed by over-correction of glasses indicating inadequate correction. However, since the glasses were chosen so as to reveal as emmetropia subjectively or objectively and no complaint was made wearing with them on for 30 minutes as stated before in experimented condition, there were, as a result, no glasses which fit the patients in this group, namely, it

might be supposed that choice of the most adequate glasses was difficult when these abnormalities appeared in accommodative function. And prolongation of relaxation time before loading was supposed as hypertonic condition of ciliary muscles. This was also proved by remarkable reduction of relaxation time with instillation of atropine, accommodation becoming emmetropia-like, and remarkable tendency of recovery, therefore, was observed.

Type II, showing almost the same change with Type I before londing, demonstrated shortening of relaxation time by correction with glasses, while it prolonged with repeated measurements.

Since accomondation of Type II was not cured so well as Type I by instillation of atropine, accommodative function of Type II might differ fundamentarily from Type I.

Then, in Group B which showed no changes in relaxation time by repeated measurements and was supposed to be fixed myopia, there were some cases which revealed the same change with Type II with correction of glasses. This was Type III. This type was divided into two subtypes according to the result of instillation of atropine; the one was Type III-1 which snowed the tendency of recovery as Type II and the other was Type III-2 which showed little effect of atropine. From these facts, Type II was alike with Type III-1 and was in transformable state to Type III, while Type III-2 resembled comparatively to Type IV.

The accommodation of Type IV changed little by correction with glasses or by instillation of atropine. Type IV was supposed to be manifest myopia.

From these arguments, Type II may transform to manifest myopia. When subjects in Type I and Type II are applied with correction of convex glasses remarkable fluctuation of visual acuity and of refraction were observed and from this results it was proved that these types are identical with so-called pseudomyopia.

Though there have been many different opinions concerning pseudomyopia by several authors, general agreement has been presented that most of pseudomyopia are caused by the disorder of accommodative function.

Type II may be regarded as the so-called pseudomyopia from many findings in this experiment, but shows some changes like Type III-1, and so this Type II may be supposed to be a first stage of myopia. On the contrary, Type I is supposed as genuine pseudemyopia.

In type V, accommodation as is seen in emmetropia is observed by correction of glasses, but is not influenced by instillation of atropine, and also, the change by myopia is found in the fundus. From these facts, this type shows myopia related to the axial length of the eye.

In comparison with type V whose chief causes are axial changes, principal changes in type III and IV may be caused by abnormalities of accommodative functions. Although in these type disorders of accommodative function or resulted changes of crystalline lens may play important roles, for, atropine affects in some degree to Type III-2 and in

Group A, the so-called pseudomyopia with the healing tendency and there exists Type II which is transformable to Types III-1, Type III-2 and to Type IV.

Repeatedly, though there exist the changes of crystalline lens or of axial length of the eye as in the Type V, changes which are revealed in Type II—IV may be effectuated chiefly by accommodative function. As shown above, myopia is caused by anomaly of accommodative functions, changes of crystalline lens and of axial length of the eye independently or intricately each other. As far as the present experiment is concerned, however, it is supposed that the possibility of healing exists in the myopia in which anomaly of accommodation occurs first. From the fact that this kind of myopia has healing tendency, accommodation as well as refraction should be examined when the myopic glasses are determined.

From the results of the present study, a key for resolution of the causality of myopia would be given and it may contribute to the therapy and prevention of myopia.

Summary

104 cases representing myopia or the so-called pseudomyopia were measured with their accommodative function, using Hagino-Suzumura's Autographic Asthenopiameter.

The results were as follows:

In every measurement, they represented characteristic changes in accommodation and from the results they were classified into five types. By further analysis, formerly supposed pseudomyopia which showed some fluctuations of accommodation, were divided into two types, namely, the one which was truly supposed pseudomyopia, and the other which might be thought to be transformable form to myopia. And also, when loadings were applied to those which did not show any fluctuations of accommodation, three types were observed; namely one which showed further fluctuation in accommodation, one which showed no change, and one in which anomaly of accommodative function was unable to be detected.

From these results, it was concluded that accommodative function might play an important role in the causality of myopia, and that in the correction of glasses, determination of two aspects, namely refraction and accommodation, would be indispensable.

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AN EXPERIMENTAL STUDY ON THE ETIOLOGY OF UVEITIS IN RELATION TO STRESS

Ichizo IKEDA

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It is well known that iridocyclitis is caused or induced by cold, overwork, sleeplessness and other conditions which are considered to be stressors of Seiye[1]. Thus it is reasonably assumed that these stressors may induce iridocyclitis giving rise to General Adaptation Syndrome (G-A-S) in the host, and the assumption is confirmed by statistical observation on uveitis patients. With regard to G-A-S, laboratory examinations (blood analysis, Thorn test, urinary 17-KS measurement, Rumpel-Leede test, etc.) revealed abnormalities or lability of adrenocortical function in many cases of uveitis.

But it is established that infections, e.g., tuberculosis, toxoplasmosis, syphilis, allergy, e.g., lens induced uveitis, hypersensitivity to toxins, and trauma cause uveitis. In the present experiment studies are made to decide if stress alone will cause uveitis.

Selye demonstrated that pro-phlogistic hormones (e.g., DOCA) caused rheumatoid arthritis, periarteriitis nodosa, etc. in albino rats. In man rheumatoid arthritis is oftern accompanied by uveitis; periarteriitis nodosa in the choroid is frequently observed. These findings suggests the possibility of occurrence of uveitis due to the stress independently of other causative agents.

The present paper deals with clinical and histological studies made on the possibility of occurrence of uveitis following prolonged administration of DOCA to albino rats and on the effects of DOCA and other various stressors on the experimental uveitis of albino rabbits.

Experiments

Changes of the uvea of albino rats following prolonged administration of DOCA (Experiment I).

Method. Ninteen of 25 albino rats which had been unilaterally nephrectomized were administered DOCA from the day of nephrectomy, and other animals were used as control. The animals were ingested with dried food staffs and 1% saline solution. The method, duration and dosage of administration are shown in table 1. Biopsy of organs and histological examination of the eye ball were made following sacrifice of the animals after a certain period of observation of general conditions and ocular signs.

Table 1. Method, Duration and Dose of Administration of DOCA (Experiment I)

Rat No.	Unilateral Nephrectomy	High NaCl. Diet	DOCA Administration		Experimentation Days
			Method	Total Dose (mg.)	
1	+	+	—	—	89
2	+	+	Syncorta i.m. (1 mg./day)	93	94*
3	+	+	〃	88	89
4	+	+	〃	88	89*
6	+	+	〃	88	89
7	+	+	Cortiron Pellet (50 mg.)	50	112*
8	+	+	Cortiron i.m. (1mg. ×15 days, 2.5 mg.×98 days)	260	113
9	+	+	Cortiron Pellet (50 mg.)	50	60*
10	+	+	—	—	113
11	+	+	Cortiron i.m. (1mg.×15 days 2.5mg.×98 days)	260	113
12	+	+	〃	260	113
13	+	+	〃	260	113
14	+	+	—	—	113
15	+	+	—	—	113
16	+	+	—	—	113
20	+	+	Cortiron i.m. (2.5mg./day)	220	101
21	+	+	〃	195	88
22	+	+	〃	135	62
23	+	+	〃	195	88
24	+	+	〃	125	57
25	+	+	〃	235	108
26	+	+	〃	220	101
27	+	+	〃	235	100
28	+	+	—	—	114
29	+	+	—	—	114

* Died of pneumonia; other animals were killed by air embolism.

Clinical findings. The DOCA administered group and the control group showed no pathological signs throughout the time of observation.

Biopsy. Two of 12 albino rats which had had DOCA intramuscularly injected showed characteristic periarteriitis nodosa of the mesenterium (Fig. 1 and 2). Neither

the animals nor the controls showed gross pathological findings except for a few animals which died during the experiment revealing pulmonary hemorrhage and peritoneal adhesion.

Fig. 1. Periarteriitis nodosa of the mesenterium. (Experiment I.)

Fig. 2. Periarteriitis nodosa of the mesenterium. Histological picture. (Experiment I.)

Histological changes of the eye ball. The control group showed no marked changes except for slight retinal edema and subretinal serous exudate observed in 2 cases (No. 1 and 2). Cell infiltrations were not demonstrated.

In the DOCA administered group a marked chronic exudative uveitis was observed in 7 of the 15 animals histologically examined; in the anterior chamber there was observed in a considerably large quantity granular substances on the posterior surface of the cornea which consisted mainly of small round cells (lymphocytes), a few mononuclear cells, and polynuclear leucocytes (Fig. 3). The iris and the ciliary body showed considerably marked hyperemia with perivascular infiltration with mononuclear cells and polynuclear leucocytes. There was also observed a marked serous exudate.

The retina was thickened showing undulation due to a marked subretinal exudation with cell infiltration (Fig. 4). The vitreous cavity showed serous exudate in a considerably large quantity. There were observed epitheloid cells.

The choroid was thickened due to serous exudation containing a slight mononuclear infiltration (Fig. 5).

Fig. 3. Cellular deposits on the posterior surface of the cornea. (Experiment I.)

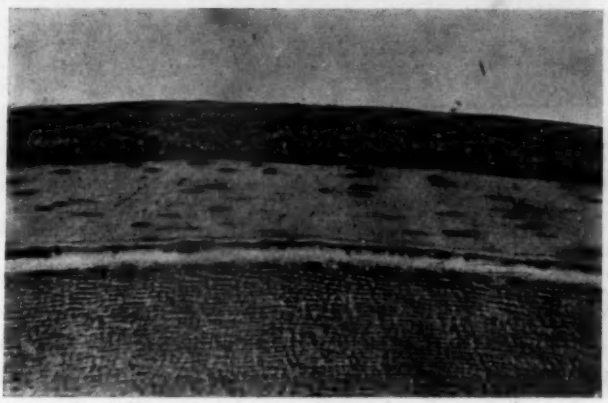
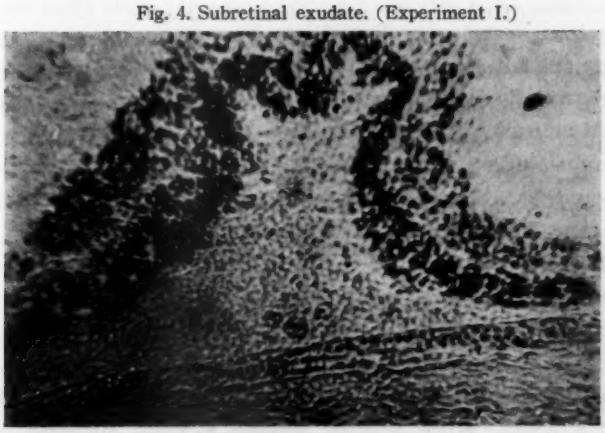


Fig. 4. Subretinal exudate. (Experiment I.)



The cases studied showed considerable individual differences in the degree of the changes mentioned above. With regard to localization of the lesion in the eye ball some cases showed marked changes mainly in the posterior part. While others showed localized changes in the anterior part or changes in both the anterior and posterior regions. No relationship was observed between these changes and periarteriitis nodosa of the mesenterium.

Effects of DOCA on horse serum uveitis (Experiment II).

Fig. 5. Cell infiltration in the choroid. (Experiment I.)

Method. One cc. of fresh horse serum was subcutaneously injected to 26 male albino rabbits weighing 2-3 kg. every other day (total 6.0 cc.). Two weeks after the last injection 0.1 cc. horse serum was intravitreously injected. In order to prevent bacterial infection a neomycin and achromycin solution was instilled into the conjunctival sac starting three days before the intravitreous injection throughout the experimental period.

Twenty six animals tested were divided into 4 groups:

Group I: three animals. As soon as a provocative injection was started, 40 mg. of DOCA was intramuscularly injected every other day throughout the observation period.

Group 2: eight animals. Starting three days before the intravitreous injection, 20 mg. of DOCA was intramuscularly injected every day.

Group 3: six animals. Starting three days before the intravitreous injection, 25 mg. of DOCA was subconjunctivally injected. The injection was given only to the right eye, the left eye being used as control.

Group 4: nine animals, used as control.

Clinical course. (1) The control group showed hyperemia and edema of the conjunctiva and ciliary injection in several hours after the provocative injection. Edema and hyperemia of the iris and dilatation of choroidal vessels with marked chemosis were observed in about 6 hours. Some animals showed a slight turbidity of the anterior chamber. But vitreous opacity was not marked, fundus oculi being clearly visualized ophthalmoscopically. In 24 hours eye lids were swollen and hyperemic. The discharge increased. The ciliary injection, and hyperemia and edema of the iris were marked. The anterior chamber grew increasingly turbid, being filled with exudate in severe cases; the fundus was no longer visualized. The vitreous opacity was marked. The cornea maintained transparency except for transient opacity in some cases. These changes reached the peak in 24-48 hours, followed by a gradual decrease. Six days

later inflammatory signs still persisted. Starting on the 14th day most animals showed no more inflammatory signs except for synechiae iridis, a deposit of fibrous elements on the iris, and, in severe cases, organized exudate in the anterior chamber. The vitreous opacity remained so marked that the fundus was scarcely vizualized, and a whitish or whitish-yellow membrane in the fundus instead of normal red reflex was observed. These changes showed considerable individual differences.

(2) With regard to a clinical picture and course there was observed no difference in systemically DOCA administered groups (group 1 and 2) and in the control group. But, if followed further, the former groups showed more marked initial chemosis, more incidence of corneal opacity and more trend of prolonged inflammation than the latter group.

(3) In group 3, in which DOCA was subconjunctivally injected, there was observed no effect of local DOCA administration on clinical signs and course of uveitis, though the degree of the inflammation of the anterior segment was dependent on dilution of horse serum used for provocative injection.

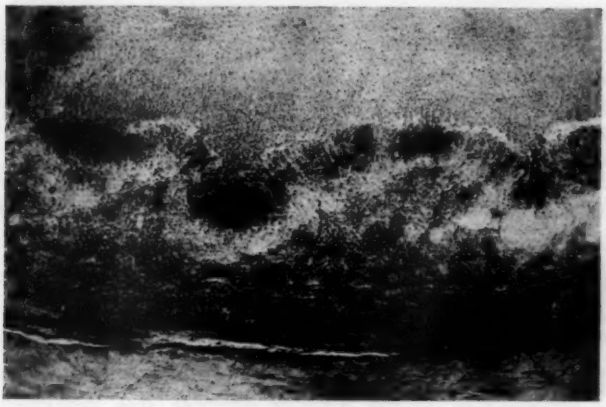
Histological findings of the eye ball. (1) Non-specific exudative inflammation was a main change in the anterior chamber, uvea, vitreous body and retina in the control group. Each individual showed same type of inflammation. Various kinds of infiltration cells were observed depending on the observation period (6-28 days after the provocative injection). In the early stage polymorphnuclear leucocytes were mostly observed, lymphocytic and plasma cell infiltrations were mainly observed in the 14 day stage. This stage occasionally showed large mononuclear cells resembling fibroblasts and epitheloid cells. Such inflammatory changes were observed most markedly in the internal limiting membrane, where a thick zone of plasmatocytic and lymphocytic infiltration was found. The cells were edematous showing no clear structure. The later the stage, the higher the degree of retinal destruction. Sometimes the retina was found detached, and the subretinal space was filled with exudate containing plasma cells, mononuclear cells and lymphocytes.

(2) The DOCA administered groups showed no essentially different histological picture from the control group. But systemically administered groups (group 1 and 2) showed from the early stage much larger area infiltrated with lymphocytes and plasma cells instead of polymorphnuclear leucocytes than the control group in the same stage. Epitheloid cells appeared in a relatively early stage and in a large quantity in the space between the choroid and the retina. Destruction of the retinal tissue was marked (Fig. 6).
Effect of stressors and DOCA on the incidence of primary hypersensitive horse serum uveitis and contralateral uveitis (Experiment III).

The third experiment was performed to study the effects of stressors and DOCA on the experimental uveitis.

One to two weeks after 0.1 cc. of horse serum was injected into the vitreous cavity of one eye of rabbits, there occurred uveitis on the same side (primary anaphylactic

Fig. 6. Destruction of the retina and the choroid. (Experiment II.)



uveitis). When a large dose of horse serum is intravenously injected one to three weeks after the intravitreous injection, occurrence of contralateral uveitis was histologically evidenced.

Method. Fresh horse serum (0.1 cc.) was injected into 30 eyes of 24 male, albino rabbits weighing two to three kg.

Group 1: adrenaline was intravenously injected every day from the day of intravitreous injection (five rabbits, eight eyes).

Group 2: electroshock was given every day from the day of intravitreous injection (six rabbits, eight eyes).

Group 3: electrostimulation of the ear lobe was given every day from the day of intravitreous injection (four rabbits, five eyes).

Group 4: 2.5 mg. of DOCA (cortiron, intravenous) was subconjunctivally injected every day starting three days before intravitreous injection of horse serum (three rabbits, three eyes).

Group 5: control group (intravitreous injection of the horse serum alone was performed with no subsequent treatment).

Clinical findings and course of ocular changes. (1) Primary anaphylactic uveitis:

(a) In the control group there appeared a slight ciliary injection and hyperemia and edema of the iris on the homolateral side from the 10th day, mostly on the 12th day after the intravitreous injection. About this time or later most cases showed exudate in the anterior chamber which adhered to the pupillary edge and the anterior surface of the crystalline lens. Two to three days later, inflammatory signs subsided. Changes of the anterior segment was followed by vitreous opacity for 1-2 days, which increased to some extent in a few days and then became stationary. In 20 days, inflammatory signs in the anterior segment almost disappeared leaving slight synechiae, but the vitreous opacity still persisted.

(b) Group 1 showed inflammatory signs such as ciliary injection and hyperemia of the iris 5-9 days after the intravitreous injection. All the cases showed more marked change than the control. Some of them showed petechiae on the iris. Vitreous opacity appeared earlier and was more marked than in the control cases. A course after the appearance of irritative signs was almost the same as in the control group and was never found prolonged. Observations in group 2 and 3 were almost the same as in group 1, but the appearance of uveitis was slightly retarded over group 1. When compared with the control group all experimental groups showed inflammatory signs earlier and in severer degree. An animal of group 3 (No. 42) showed bleeding from the iris immediately after electrostimulation of the ear lobe.

Group 4 revealed no differences in clinical findings between the experimental eye and the (contralateral) control eye or eyes of the control group.

(2) Contralateral uveitis:

Immediately after intravenous injection of 20 cc. of horse serum, the same eye, which had been inactive, showed a slight ciliary injection, hyperemia of the iris and a slight turbidity of the anterior chamber which was cleared in a few days. On the contralateral eye of the control group no clinical changes were observed throughout the observation time.

With regard to the experimental groups some cases of group 1 and 2 showed a slight ciliary injection, and hyperemia of the iris for a few days, while no cases in group 3 and 4 showed such clinical changes found in the control group.

Histological findings. (1) Primary anaphylactic uveitis:

(a) The control group mostly showed chronic inflammatory changes. Though there were observed in the anterior chamber lymphocytes and large mononuclear cells with serous exudate, the cellular component was generally rather scanty. The iris and the ciliary body contained an infiltration of plasma cells, small round cells (lymphocytes), and they showed hyperemia aud edema of tissue. Some cases showed a fibrinous exudate between the iris and the crystalline lens. In the vitreous body there were observed fibrin and cellular elements (mainly round cells and a few polymorph- and mononuclear cells). These cell infiltrations were generally found in the layer adjacent to the retina. The choroid was hyperemic, and infiltrated by plasma cells and small round cells. Its inner layer contained epitheloid cells. The fluidal exudate between the choroid and the retina contained infiltrated plasma cells, lymphocytes and mononuclear cells. The retina showed the same cell infiltration as the choroid.

(b) Experimentation groups showed no essential difference in the quality of infiltrated cells as compared with the control group. Generally speaking, however, it was noted that in group 1, 2, and 3 hyperemia and edematous changes of ocular tissues and cell infiltration in the vitreous body and retina were more marked than in the control group (Fig. 7 and 8). Group 4 showed no difference from the control group.

(2) Contralateral uveitis:

Fig. 7. Primary anaphylactic uveitis: cell infiltration in the retina and the choroid. (Experiment III.)

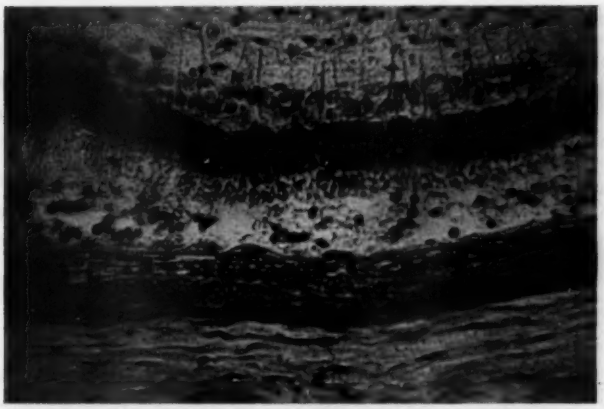
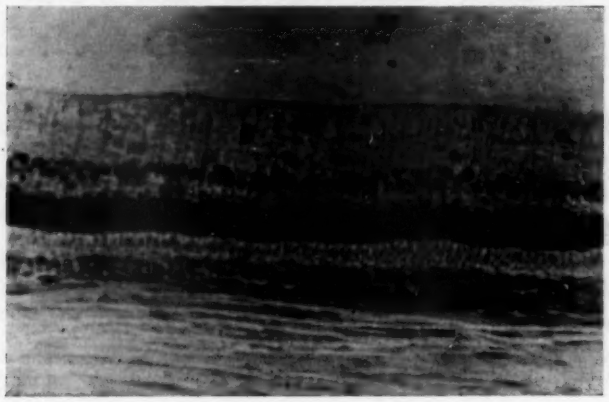


Fig. 8. Primary anaphylactic uveitis: control. (Experiment III.)



(a) Main changes in the control group were hyperemia, a slight edema and cell infiltration in the uvea. The cell infiltration, mainly of lymphocytes, occasionally with a few polymorphnuclear leucocytes, occupied the perivascular space of the choroid, ciliary body and iris in most instances, and the outer layer of the retina in some instances. All these changes were very slight.

(b) In the experimentation groups (group 1, 2 and 3) the above-mentioned changes were more marked than in the control group. The difference was not essential but quantitative (Fig. 9 and 10). Group 4 showed no difference from the control group.

Summary and Conclusion

1. Two of 19 albino rats which had been unilaterally nephrectomized and ingested with high NaCl and administered DOCA, developed paeriarteriitis nodosa of mesenteric

Fig. 9. Contralateral uveitis: cell infiltration in the iris and the cliary body. (Experiment III.)

Fig. 10. Contralateral uveitis: control. (Experiment III.)

arteries, while no clinical signs of inflammation of the uvea were grossly confirmed. However, there was histologically observed an occurrence of uveitis which did not show specific changes as seen in rheumatoid arthritis or periarteriitis nodosa but non-specific exudative changes.

2. A large dosage of DOCA was systemically administered to rabbits before and after the occurrence of experimental uveitis, whose nature of exudative inflammation was not influenced by DOCA clinically or histologically, though DOCA administered cases clinically showed a slight enhancement and prolongation of the inflammation. Histologically a marked deterioration of the retina and the early appearance of cell infiltration were suggestive of a chronic inflammation. Local administration of DOCA, however, showed no effect on the inflammation.

3. Stressors, continuously applied to rabbits, accelerated the appearance of primary

anaphylactic uveitis and intensified the severity of the uveitis ; the contralateral uveitis which was demonstrable only by histological examinations, was so enhanced that it was clinically observable.

4. These findings suggested that a possibility of the occurrence of uveitis following adminstration of a large dosage of DOCA in albino rats which had been conditioned. It is questionable, however, that those inflammatory changes mentioned above are attributable to the effect of DOCA. Experimental intervention, microbal infection and allergic changes following experimentation must be taken into consideration, because the control group which has been unilaterally nephrectomized and ingested with high NaCl without administration of DOCA showed a slight exudative uveitis which was observed in a high percentage in those animals which histologically showed pneumonia and died during experimentation. Nevertheless, it is histologically confirmed that systemic administration of DOCA accelerated inflammation of the uvea.

5. It is generally accepted by many investigators (Selye et al.) that DOCA accelerates inflammatory, especially granulomatous reactions. Aoki[2] observed the effect of DOCA on Arthus phenomenon and found that leucocytes, plasma cells and vascular reaction decreased in the presence of a marked edematous change, Aoki was skeptical about the inflammation accelerating action of DOCA and was of an opinion that inflammatory reaction was not directly influenced by DOCA, but was apparently accelerated following modification of reaction of the host due to changes in water metabolism which had been brought about by administration of DOCA.

The results of experiment II in which DOCA was given to animals which had been sensitized with horse serum, showed an early appearance of conjunctival edema and corneal turbidity ; no difference was observed in inflammatroy signs in the acute stage between experimental animals and control animals. These findings coincides with those of Kuratomi[3] who found that locally administered DOCA showed no effect on the tuberculous lesion in animals with experimental tuberculosis but resulted in an acceleration of inflammatory changes of the cornea and a delay of healing.

Thus, we could not reach clear cut conclusion from experiment II, but the results of experiment I showed that there was a possibility that DOCA made the host liable to give rise to inflammation, though evidence was not established if DOCA directly accelerated an acute inflammation.

6. It is clearly demonstrated in experiment III that continuously applied stressors accelerate the appearance of uveitis. This action of stressors seems to have no relationship with the action of DOCA which induces a localized inflammation, because, in experiment III, DOCA showed no local accelerating action on experimental uveitis. Uveitis might have been accelerated by systemic "umstimmung" of the autonomic nervous system and vascular system such as non-specific choroid reaction (Komi[4]) which is observed in stress.

To our disappointment it is not evidenced on the basis of our experiments that stress can be a cause of uveitis.

It is confirmed on the basis of the results of the present experiments that stress plays an important role as disposing and provoking factor of uveitis. Individuals who are continuously exposed to stress may manifest clinical signs of uveitis, if any causative agent of uveitis (e.g., bacteria) is added to them, when otherwise they would remain asymptomatic (disposing factor). Stressors may facillitate the occurrence of uveitis in cases which have been left asymptomatic in the presence of causative agents (e.g., focal infection) lying latent (provoking factor).

The occurrence of uveitis following systemic administration of DOCA is a very interesting problem in view of the stress theory, but further study is required before a final conclusion is obtained.

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STUDIES ON THE SUBRETINAL FLUID REPORT II. REFRACTIVE INDEX AND Cl^-, Na^+ AND K^+ CONCENTRATIONS OF SUBRETINAL FLUID IN SPONTANEOUS RETINAL DETACHMENT

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Introduction

A report on the refractive index and on the paper electrophoretic pattern of subretinal fluid in spontaneous retinal detachment has been presented in Report I and in this report the results of the study made on the refractive index and on the Cl^-, Na^+ and K^+ concentrations will be presented.

Method

Method of obtaining subretinal fluid.

The procedure described in Report I (J.J.O. Vol. 3, P. 23) was employed, but all the instruments used in the procedure were washed in distilled water following ion removal. The standard amount obtained was at least 0.3 cc, the minimum required for the various determinations.

Determination of the refractive index.

As in Report I, Abbe's refractometer was used for the determination.

Determination of Cl^- concentration.

By Rusznyak's chlorine microdetermination method, the Cl^- concentration was computed.

Determination of Na^+ and K^+ concentrations.

Lange's model V flame photometer was used with air pressure at 0.5 atmosphere and with the sensitivity adjustor placed at Determination of Cl^- concentration.

0.1–0.2 cc of the fluid to be tested was diluted 100 fold. 8.185 g of NaCl and 0.3226 g of KCl was dissolved in 100 cc to make a standard serum stock solution of Na^+ 140 mEq/l, with which standard solution of desired concentration were prepared.

Results

Cases on which determinations were made.

As shown in Table 1, determinations were made on 12 cases (12 eyes). However, for Case No. 1 fluid was obtained again on the 18th day and, therefore, determination were performed on a total of 13 speciments.

Table 1. Cases of Subretinal Fluid Determinated

Case	Retinal Detachment			Operation	Post-Operative Course
	State	Detection of Tear	Duration		
(1) Y.N. 29 ♀ L	Vesicular	not found	8 Years	P.(2 times)	
(2) S.H. 74 ♀ L	Vesicular	found	10 Days	D.S.	Reattachment
(3) J.S. 57 ♂ L	Vesicular	found	12 Days	D.	Reattachment
(4) S.K. 45 ♀ R	Wrinkled	found	4Months	C.	Failure
(5) M.S. 48 ♀ L	Vesicular	found	4.5 Years	D.	Failure
(6) K.Y. 36 ♂ R	Vesicular	found	3 Years	D.	Reattachment
(7) T.T. 23 ♂ R	Wrinkled	not found		P.	
(8) Y.I. 19 ♂ R	Wrinkled	not found	2 Years	S.	Failure
(9) K.S. 57 ♀ L	Wrinkled	found	10 Days	P.S.	Reattachment
(10) K.H. 51 ♂ L	Wrinkled	found	3 Days	D.S.	Failure
(11) Y.U. 64 ♀ R	Wrinkled	found	24 Days	S.	Reattachment
(12) Y.M. 65 ♂ L	Wrinkled	not found	14 Days	P.	

Cases studied were all cases of spontaneous retinal detachment. There were no cases in which the detachment was flat in form, because aspiration of subretinal fluid was confined to cases with comparatively severe degree of retinal detachment and to cases in which retinal reattachment could not be expected by rest. Because of these reasons, the percentage with favorable post-operative course was low.

The method of computing the duration of retinal detachment and the symbols used in the "operation" column are identical to that described in Report I.

Determination results.

The results of determinations made on 13 specimens obtained from the foregoing

Table 2. Refractive Index and Ion Concentrations of Subretinal Fluid

Case	obtained Subretinal Fluid (cc.)	Refractive Index	Ion Concentrations (mEq/l)		
			Cl^-	Na^+	K^+
(1)	1.0	1.3507	108.71	123.5	5.75
(1) 2nd time	1.0	1.3370	116.86	154.8	5.30
(2)	0.4	1.3398	119.25	146.4	5.45
(3)	0.2	1.3376	121.05		
(4)	0.35	1.3470	115.57	147.0	5.80
(5)	0.35	1.3552	112.46	128.0	6.35
(6)	0.4	1.3373	121.57	151.0	5.25
(7)	0.75	1.3655	108.71	108.0	6.05
(8)	0.75	1.3576	108.37	80.5	5.25
(9)	0.4	1.3380	120.09	126.0	4.50
(10)	0.35	1.3370	125.94	169.5	5.65
(11)	0.45	1.3405	117.83	125.5	3.75
(12)	1.0	1.3410	114.66	145.0	5.0

12 cases are shown in Table 2.

As the amount aspirated from Case 3 was insufficient, determination of Na^+ and K^+ concentrations could not be made.

Discussion

Refractive index.

The finding presented in Report I that the refractive index is higher with the extension of the duration of retinal detachment was again confirmed in the study of these 12 cases.

Cl^-, Na^+ and K^+ concentrations.

As shown in Table 2, a considerable variation in the respective ion concentrations was noted in the cases. That is, the concentration of Cl^- varied from 108.37 to 125.94 mEq/l and that of K^+ from 3.75–6.35 m Eq/l.

The mean ion concentrations of six cases (Case Nos. 2,4,9,10,11,12) whose duration of retinal detachment was less than 4 months were obtained and compared with values reported in literature for serum and vitreous humor (Table 3). The respective ion concentrations of subretinal fluid were higher than that of serum and the mean Cl^-, K^+ concentrations of these cases were close in value to that of vitreous humor.

The relation between refractive index and Cl^-, Na^+ and K^+ concentrations.

A tendency was noted for the ion concentrations of Cl^- and Na^+ to be higher as the

Table 3. Ion Concentrations of Subretnal Fluid in Spontaneous Retinal Detachment (6 cases whose duration was less than 4 months), Serum and Vitreous

	Cl^- mEq/l	Na^+ mEq/l	K^+ mEq/l
Subretinal Fluid (mean value)	114.66 ~125.94 (118.89)	125.5 ~169.5 (143.2)	3.75 ~5.80 (5.03)
Serum (mean value) [Shibata]	100~106	134~146 (140.4)	3.6~5.2 (4.0)
Vitreous [Duke-Elder]	119.08	118.7	4.92

refractive index lowers. This contradicts the finding of Magitot who made determinations on the chloride and protein concentrations of subretinal fluid in spontaneous retinal detachment. The present results indicates a quantitative inverse correlation between Cl^- concentration and protein concentration which plays a major role in refractive index and this property is in accord with the relation noted in aqueous humor and vitreous humor by Duker-Elder. No correlation was noted between K^+ concentration and refractive index.

Relation between duration of retinal detachment and ion concentration.

As can be expected from the foregoing, a tendency was noted for the Cl^- and Na^+ concentrations to be lower in cases where the duration of detachment was longer.

Mutual relation among concentration of Cl^-, Na^+ and K^+.

A quantitative balance was noted between Cl^- concentration and Na^+ concentration. That is, in cases where the Cl^- concentration was low, the Na^+ concentration was also low and this was true in the opposite case. However, it was noted that no correlation existed between Cl^- concentration and K^+ concentration or between Na^+ concentration and K^+ concentration.

Relation between state of retinal detachment and ion concentration.

No specific correlation could be observed. Inasmuch as the number of cases was small with no cases of flat retinal detachment, conclusive statement regarding this relation will be deferred.

Relation between postoperative course and ion concentration.

It was difficult to note a definite relation, but except for one case (Case No. 10) a tendency was observed for the reattached cases to show a high Cl^- concentration.

Summary

Determinations of the refractive index, Cl^- concentration (by chemical analysis) and Na^+ and K^+ concentration (by flame photometry) were made on 13 specimens of subretinal fluid aspirated from 12 cases (12 eyes) with spontaneous detachment. A

study was made on the relation between the results of these determinations and the clinical findings and the following results were obtained.

1. The finding presented in Report I that the refractive index is higher with the extension of the duration of retinal detachment was re-confirmed.

2. The concentration of Cl^- varied from 108.37 to 125.94 mEq/l, that of Na^+ from 80.5 to 169.5 mEq/l and that of K^+ from 3.75-6.35 mEq/l.

3. A quantitative inverse correlation was noted between Cl^- and Na^+ concentrations and refractive index (protein concentration).

4. No correlation was seen between K^+ concentration and refractive index.

5. A quantitative balance was noted between Cl^- concentration and Na^+ concentration, but such balance was not observed between Cl^- concentration and K^+ concentration or between Na^+ concentration and K^+ concentration.

6. It was observed that Cl^- and Na^+ concentrations were lower in cases where the duration of retinal detachment was longer.

7. No definite relation was noted between the postoperative course and ion concentration except that reattached cases almost invariably showed a high Cl^- concentration.

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STUDIES ON HETEROKERATOPLASTY

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Introduction

Not a few individuals suffer from either blindess or marked visual disturbance merely due to the development of corneal opacity even in the absence of any abnormality of the fundus. It is also well known that these patients may regain their normal sight by transplantation of their corneas. The practice of corneal transplantation, however, is best with three serious handicaps: 1, difficulty, encountered especially in Japan, in obtaining the eye-balls from cadavers soon after death, because of religious,

sentimental or other social prejudices; 2, shortness of the period of preservation even when the eye-balls are available; and 3, uncertainty of the time when they are obtained. These difficulties make it almost impossible for the patients to be benefited by corneal transplantation at proper time and desired place. These disadvantages can be overcome by the use of animal corneas under proper specifications.

Transplantation of animal cornea, or heterotransplantation, has long been tried but most of the attempts have ended in failure. More recently, this problem has received special attention by Müller, Maumenee, Basu, Ormsby, Lieb, Tsutsui and other investigators, although their studies covered only the fundamental aspects of the procedure without any endeavors to apply to human eyes. Since 1956 the author in cooperation with Kamata, Senoo, Hirakawa, Nonaka, Kogure, Arai and others, has been engaged in basic as well as clinical researches using the corneal tissue of domestic fowls, transplanting it to the lamellar portion of human eyes. The results of studies have been fairly satisfactory and the procedure is described in the following paragraphs.

Basic Experiment

Before undertaking heterotransplantation, it is necessary to make thorough and careful studies of the corneas of various animals for the purpose of choosing proper donors. The fundamental considerations were first given to the histological and serological aspects of the tissues concerned.

Histological examination:

The first prerequisit is to find a suitable doner animal from which the corneal tissue may be secured with relative ease. The corneas of monkeys, cattle, horses, swines, cats, rabbits and domestic fowls were investigated, using human corneas as controls. The tissue was fixed in formalin and either paraffin sections or frozen sections were prepared. These were stained by hematoxylin-eosin, PAS or Mallory stain for comparative histological examination. Without resorting to irrelevant details, only the noteworthy feature will be here described from the standpoint of keratoplasty.

The most noticeable finding is that the spaces filled with corneal tissue fluid in the parenchyma are extremely different from each other according to the animal species. As to the width and number of the spaces in the parenchyma, the animals examined may be divided into two groups:

(1) Those with extremely narrow spaces, making the corneas to appear compact (humans, rabbits, cattle, horses, dogs, swine and cats). (Fig. 1. A,B)

(2) Those with numerous wide spaces (monkeys, domestic fowls). (Fig. A,B) The corneas of animals belonging to the latter group have apparently no vascular supplies, and the nutriments are believed to be derived from the tissue fluid. It is accordingly supposed that this condition favers a good circulation of tissue fluid and ensures on active state of metabolism. Histologically, therefore, this group of animals is considered

Fig. 1 (A). Rabbit cornea.

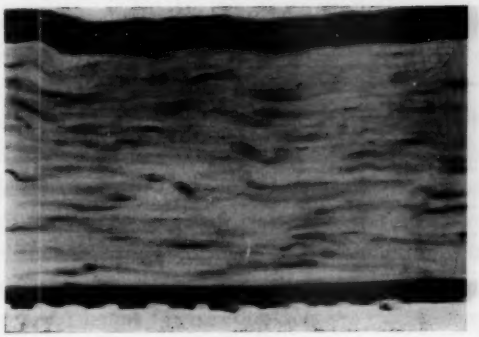


Fig. 1 (B). Rabbit cornea.

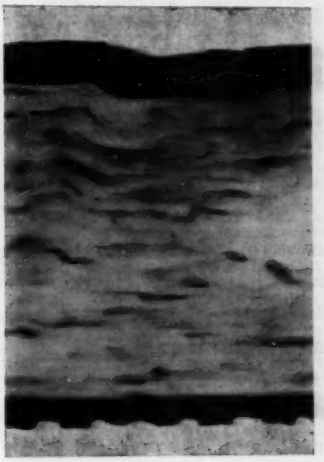


Fig. 2 (A). Fowl cornea.

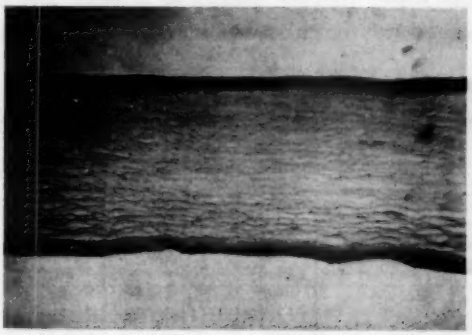
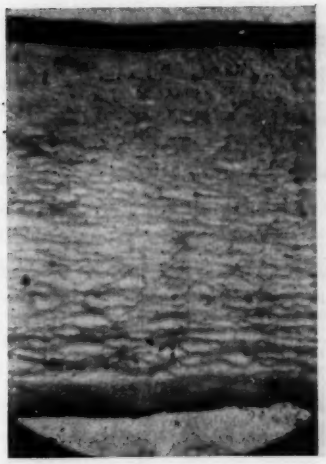


Fig. 2 (B). Fowl cornea.



to be best adapted to heterotransplantation.

Biochemical consideration :

In the study of heterotransplantation of corneas the question as to the reaction of foreign proteins has long been debated. In order to determine the protein composition of corneal tissues obtained from rabbits, fowls, dogs, monkeys and humans, the free amino acids in the protein hydrolysates were analyzed by the paperchromatographic method (Table 1). One significant finding was that the human corneas and those

of fowls were remarkably similar in the total amino acid contents, giving the values of 316,0γ and 319,0γ respectively. However, the human corneas contain slightly more sulfonic amino acids and less tyrosine, as compared with those of monkeys, dogs, cats and rabbits. It is reasonable to state that the corneas endowed with active metabolism and vitality are most desirable when they are to be used as grafts in heterotransplantation.

Using fowls, representing the first of the histologic group above referred to, and rabbits, cats and dogs, typifying the second, the oxidative and glycolytic processes of the corneas were measured by Warburg manometer (Table 2). It was then found that the fowl corneas are highly active in oxidation and glycolysis, being nearly three times as active as those of rabbits and other animals, indicating a state of active metabolism associated with the presence of wide spaces in the parenchyma. Some investigators insist that the corneal grafts with active metabolism are not necessarily suited for transplantation, but that those of durable viability should be preferred, even if they show low metabolism. In order to clarify this point, a comparative durability test was made between the fowl corneas and those of rabbits by the use of Warburg manometer (Table 3). The results obtained indicated that both oxidation and glycolysis continued for as long as 16 hours in the fowl corneas, whereas these ceased within 10 hours in those of rabbits. These observations suggest that the corneas of domestic fowls are endowed with higher metabolic activity and longer viability than those of other animals. It is, therefore, considered that the fowl corneas are the most suitable as corneal grafts in heterotransplantation.

Table 1. Amino Acid Group (γ/500γ) in Hydrated Corneal Protein

	Amino Acid Group	Monoamino Monocarboxylic Acid	Amino Acid with Surfur	Monoamino Dicarboxylic Acid	Basic Amino Acid	Aromatic Amino Acid	Heterocyclic Amino Acid
Normal Cornea	Rabbt	235 (56.6)	28.5 (6.9)	48 (11.6)	61 (14.7)	32 (7.7)	11 (2.6)
	Domestic Fowl	180 (56.4)	4.3 (13.5)	32 (1.00)	53 (16.6)	0 (0)	11 (3.5)
	Dog	194.5 (44.0)	5.0 (11.7)	35 (8.0)	86 (19.7)	62 (14.2)	9 (2.6)
	Human Adult	193 (60.0)	4.8 (15.2)	22 (7.0)	37 (11.4)	Trace (0)	16 (5.6)
	Cat	187 (51.3)	2.1 (5.6)	23 (6.3)	65.5 (19.0)	34 (9.8)	34 (9.8)
	Monkey	215 (62.7)	2.7 (8.0)	45 (12.9)	28 (8.2)	0 (0)	28 (8.2)

Examination by tissue culture.

Tissue reactions are apt to occur between the recipient cornea and the donor graft in heterotransplantation of corneas. For the purpose of observing these reactions in the living body from the dynamic point of view, a series of tissue culture experiments was undertaken, simultaneously employing the corneal tissues of fowls and rabbits. The method utilized the usual technic of tissue culture. The finely chopped pieces (approximately 1×1 mm in size) of the corneas removed from these two species were placed

Table 2. The Metabolism of the Cornea of Different Animals

	Qo_2	$Q_M^{O_2}$
Rabbit	−1.22	+0.74
Dog	−1.20	+0.73
Cat	−0.95	+0.63
Horse	−0.71	+0.28
Cattle	−0.69	+0.26
Pig	−1.04	+0.36
Chichen	−2.95	+2.29
Carp	−1.49	+0.47

Table 3. Durability Test of the Cornea

Rabbit's Cornea

	Qo_2	$Q_M^{O_2}$		Oo_2	$Q_M^{O_2}$
1 H	−1.25	+0.94	6 H	−0.52	+0.61
2 〃	−1.21	+0.81	7 〃	−0.50	+0.54
3 〃	−1.10	+0.90	8 〃	−0.47	+0.66
4 〃	−0.92	+0.68	9 〃	−0.44	+0.83
5 〃	−0.68	+0.72	10 〃	−0.41	+0.75

Chicken's Cornea

	Qo_2	$Q_M^{O_2}$		Qo_2	$Q_M^{O_2}$
1 H	−2.80	+2.36	9 H	−1.42	+2.34
2 〃	−2.78	+2.55	10 〃	−1.28	+2.11
3 〃	−2.75	+2.23	11 〃	−1.12	+2.03
4 〃	−2.60	+2.61	12 〃	−1.02	+2.09
5 〃	−2.33	+2.83	13 〃	−0.81	+2.35
6 〃	−2.24	+1.95	14 〃	−0.73	+2.43
7 〃	−1.98	+2.41	15 〃	−0.64	+2.84
8 〃	−1.71	+2.83	16 〃	−0.61	+2.51

side by side at the distance of about 2 mm on a culture medium prepared by mixing the fowl serum and embryonic juice. In the beginning of the experiment, it was anticipated that, when these heterogeneous grafts were cultured side by side, the proliferating cells would come into contact with other and give rise to the tissue reactions hitherto observed in corneal heterotransplantation, such as degeneration, necrosis and phagocytosis of these cells. It was, however, beyond the authors' expectation to find that no evidence of tissue reaction was obtained, as the contacting borders showed a smooth transition and no demarkation areas could be observed. From this fact it is inferred that the proper cells of the cornea exhibit no antigenic properties and that the antigenicity of the cornea may originate from the proteins contained in the fluid circulating in the corneal tissue. The proteins in the tissue fluid naturally vary according to the animal species, but the fact that no tissue reactions were observed in the present experiment was interpreted to signify that the two varieties of foreign proteins coming in contact with each other were both nourished by the same tissue fluid of the culture medium. If this assumption is true, no tissue reactions should occur when heterotransplantation is made after replacing the fluid of the donor cornea with that of the recipient cornea. Concretely, it is preferable to use the corneas with wide and numerous spaces in order to facilitate the replacement of tissue fluids. For this purpose the corneas of domestic fowls are most suitable. Since it is extremely difficult to remove the tissue fluid from the recipient's cornea, the serum may well be used as a substitute. Furthermore, in consideration of the histologic structure, metabolism and amino acid composition, it was found that the fowl corneas proved to be the most suitable donor tissue. Briefly stated, the essential steps in successful heterotrans-

plantation consist of the use of fowl cornea, immersing it in the recipient's serum for exchange of tissue fluid and its transplantation into the recipient.

Animal Experiments

A series of animal experiments was carried out in order to determine the conditions required for successful heterotransplantation based on the findings of basic studies, and at the same time observations on the antigen-antibody reactions on the part of the recipient animals were made. It was confirmed that the serum of the recipient animals was most suitable for replacing the corneal fluid, although an attempt was also made to see if there were other equally suitable or even better substitute for this purpose. Rabbits were used as recipient animals, while the fowls served as donors. The fowl corneas were first immersed in various kinds of fluid and then it was transplanted lamellarly on to the cornea of rabbits.

Observations were made on the two groups of animals treated according to two different procedures.

Group A. In the first group the fowl corneas were grafted on the corneas of rabbits involving the following differences in procedures:

(1) Without any pretreatment
(2) Immersion in the rabbit serum
(3) Immersion in physiolagical saline
(4) Immersion in Ringer's solution
(5) Immersion in formalin
(6) Immersion in fowl plasma
(7) Immersion in essential amino acid solution

Group B. In the second group the rabbit corneas removed from one eye was transplanted on to the other eye of the same animal, after the graft was immersed in various kinds of replacement fluid:

(1) Immersion in physiological saline
(2) Immersion in fowl plasma
(3) Immersion in human plasma
(4) Immersion in rabbit plasma

In all of these 11 experiments of both groups, the adhesion areas of transplanted grafts were transparent in test (2) of grous A and in (1) and (4) of group B, while all remaining grafts developed turbidity. There were also many grafts falling off due to tissue reactions. Upon close examination of the results obtained, it became clear that the heterotransplantation in Group A was successful when the donor's cornea was immersed in recipient's serum, while the autotransplantation in tests (2) and (3) in Group B proved a failure, where the corneas were immersed in heterogeneous serum. In short, it was confirmed that when the tissue fluid of the donor's cornea was the same as that of the recipient the resulting adhesions on heterotransplantation are trans-

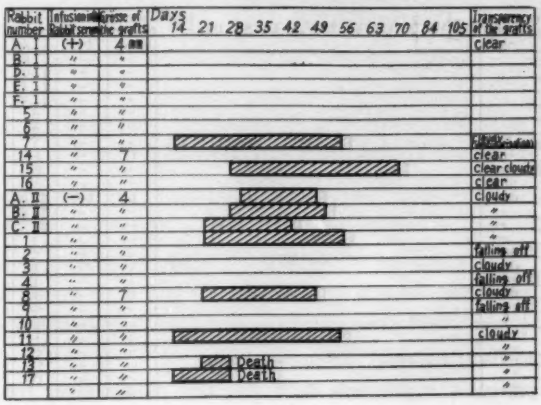
parent, just as the fundamental experiments indicated. Thus, it may be concluded that the replacement of the corneal tissue fluid is facilitated when the histologic structure of the cornea favors this procedure, the corneas provided with wide and numerous spaces being more suitable than others.

In further experiments the corneas of fowls, cats, dogs and human beings were first immersed in rabbit serum and then transplanted lamellarly on to the rabbit cornea. It was then found that only the fowl cornea was successful in producing transparent adhesion, all others being unsuccessful. This indicates again the fact that the corneal fluid could be readily replaced because of wide and numerous spaces in the fowl cornea, thus providing another proof in favore of the findings obtained in the basic experiments.

The Antigen-Antibody Reaction

Since the emergence of antigen-antibody reaction is one of the most important problems in successful heterotransplantation, the author made two series of experiments. In the first the cornea of a fowl was transplanted lamellarly without any pretreatment to the rabbit and the precipitin tests were performed at weekly intervals. The results given in Table 4 show that the antibody production was recognized within 2 to 4 weeks. the period of active production extending from 3 to 6 weeks. Clinically, the corneal grafts became cloudy in all cases, some being even sloughed off. In the second series of experiments, the grafts were immersed in rabbit serum for 24 hours, prior to transplantation (Table 4). Only in 2 instances was the antibody production demonstrable and the grafts became cloudy, but in all other cases the presence of antibody could not be detected, the adhesions remaining transparent throughout the period of observation. These findings confirm the fact that by immersing the corneas in the recipient's serum the production of antibody can be circumvented. The author is thus led to believe that the protein giving rise to the antibody may not be a part of the proper cells of the cornea but originates from the tissue fluid circulating in the cornea.

Table 4. Relaton Between the Production of Antibody and the Transparency of Grafts on the Corneal Heterografts



Clinical Cases

In the light of the findings obtained in basic and animal experiments, the author attempted transplantation of fowl corneas, immersed in recipient serum, on to the human corneas in 10 clinical cases. All these trials were fortunately successful and the details of clinical course and progress in 2 cases, accompanied by photographic records, will be here described.

Case 1.

The patient was a 14-year old female child with chief complaint of bilateral defective vision, first noted several years previously and growing steadily worse since. She was first seen in January, 1955, when her vision was recorded to be v.d.=0.01 (n.c.), v.s.=0.2 (n.c.). Aggregations of gray punctate opactities were present in the superficial layer of the cornea, particularly severe in the right eye. This was thus a typical case of Graenow's corneal opacity. The author immediately attempted lamellar transplantation of human cornea on the right eye, and her sight was restored to 0.4, while the left eye was still 0.2. About a year later, February, 1956, the patient visited our clinic again, when the examination revealed her sight to be v.d.=0.4 (n.c.), v.s.=0.01 (n.c.). The transplanted part of the cornea was transparent in the right eye, but the superficial opacity of the left eye had increased (Fig. 3). Fowl cornea was then transplanted lamellarly in the left eye.

Fig. 3. Case 1. Before operation.

Operative procedure: Under instillation anesthesia a disk-shaped button of the superficial layer of the opaque cornea, about 0.1 mm in thickness, was removed by means of a 4 mm trephine. The resulting defect was filled by inserting a disk of fowl corneal graft 4 mm in diameter, about 0.1 mm in thick, which had been preserved in the patient's serum at 4°C for 24 hours and then carefully and thoroughly washed in sterile distilled water. The graft was fixed securely by cross-suture.

Progress: The patient's sight was 0.15 for one month after the operation and the

transplanted graft remained transparent. The improvement in sight progressed to 0.4 during the 2nd month and to 0.3 during the following year. It became stationary ever since. (Fig. 4).

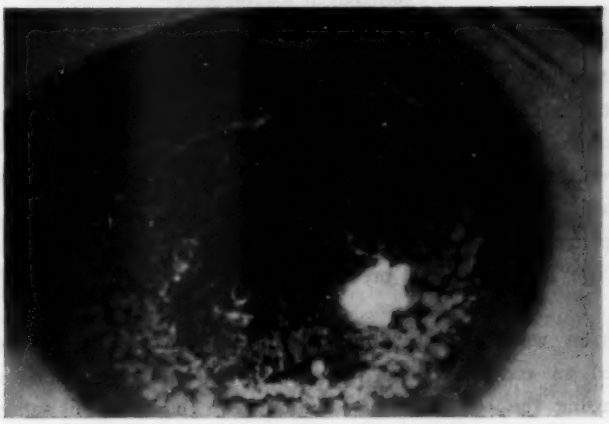
Fig. 4. Case 1. After operation.

Case 2.

A 20-year old female patient complaining of bilateral visual disturbance due to Graenow's corneal opacity began to note the sight defect since she was in the 6th grade of primary school. The condition grew increasing worse until one of her friends pointed out a white blur on her cornea.

When the patient visited the author's clinic in June, 1960, the examination revealed v.d.=0.1 (n.c.), v.s.=0.08 (n.c.). The eye-lide and conjunctive were normal. A disk-shaped opacity, about 8 mm in diameter, was found in the center of the cornea, and punctate or net-like opacities were seen scattered through the opaque area as determined by means of slit lamp microscopy (Fig. 5). These changes were noted to lie subepithe-

Fig. 5. Case 2. Before operation.



lially. The anterior chamber was normal in depth but it was difficult to examine the iris or the fundus satisfactorily.

Operative proccedure: On July 8, 1960, the transplantation of fowl cornea was made on the left eye. The graft was immersed in the patient's serum previously prepared, preserving it at 4°C for 48 hours. By means of a 6 mm trephine a piece of corneal tissue, 6 mm in diameter and 0.1 mm in thickness, was removed, and the defect was filled with a fowl corneal graft of approximately the same size, securing it by applying 4 direct sutures.

Progress: Postoperative course was uneventful. On the 15th day, the sight was 0.1 (n.c.) and the transplanted graft was transparent. On the 24th day the sight became 0.2 (n.c.) and on the 29th day it improved to 0.5 (n.c.) (Fig. 6). During the year which followed the patient's visual condition was remained practically similar.

Fig. 6. Case 2. After operation.

Serological Examination

For the purpose of confirming the results of animal experiment in regard to the absence of antibody production when heteroplantation is made with the graft previously immersed in the recipient's serum, the author investigated the serological problems concerned in human procedure. The observations are summarized as follows:

Precipitin reaction: Antigen was prepared from the serum of domestic fowl, inactivated by heating at 56°C for 10 minutes, to which 1000 : 1 NaN_3 was added. For antiserum the patient's serum, taken in early morning before breakfast, inactivated by heating at 60°C for 3 minutes, and preserved at 4°C with addition of 1000 : 1 NaN_3, was utilized.

The antiserum was used undiluted, and the paient's serum obtained before the operation served as control. A set of serial dilutions of the antigen down the 20480 was prepared using the original 1 : 10 dilution with physiological saline. The reactions

were observed for a period of 5 hours.

The results indicated a complete absence of positive reactions during the first 7 weeks postoperatively.

Further tests more sensitive than the precipitin reaction were performed by utilizing tannic acid and protein-treated hemagglutination reaction (Stavitsky). The same antigen and antiserum as those used in the precipitin reaction were employed. Tannic acid was diluted 1 : 2000 with physiological saline, while the antiserum was prepared from Group A,B human serum diluted 1 : 100 with saline. The antigen concentration was 1 : 40. Group 0 human red cells were used.

The test were made in accordance with the technic recommended by Stavitsky and the reactions were read after 3 hours, and again after 24 hours of standing at 4°C.

The results were completely negative during 1-7 weeks postoperatively, confirming the previons finding that the fowl corneal grafts, first immersed in human serum and then transplanted on to the human cornea, remains transparent and no evidence of antibody production can be obtained.

Conclusion

The results of the author's study as summarized in the present communication establish beyond any doubt that the fowl cornea is the most suitable tissue for preparing the grafts in transplantation by lamellar keratoplasty, especially after first immersing in the recipient's serum. In regard to penetrating keratoplasty, the author is engaged in still further investigations.

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EFFECTS OF HERPES SIMPLEX VIRUS ON THE TISSUE CULTURE CELLS OF THE RABBIT CORNEA

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Introduction

In the study of herpes simplex virus, the epithelium of the rabbit cornea has been widely used in vivo and in vitro[1,2,7,10] as well.

In the previous paper it was reported that herpes simplex virus caused a cytopathogenic effect on the strain of fibroblasts derived from the rabbit cornea[14]. However, the possibility that variation of susceptibility has occurred in cells after prolonged cultivations has to be considered.

The purpose of this study is to investigate the effect of this virus on primary cultures of epithelium, stroma and endothelium, and to compare the susceptibility of these cells with that of the strain of fibroblasts which are thought to have originated from the stromal cells.

Materials and Methods

Eye balls were obtained from freshly killed rabbits. A thin slice of the superficial layer was shaved off the cornea at first, then the residual corneal tissue was cut out and divided into two parts, stroma and endothelium with Descemet's membrane. Those tissues were cut into pieces approximately 1.5 to 2 mm in diameter, and two fragments of each tissue were placed on a coverslip (7×40 mm) coated with a rabbit plasmal clot. Some of the epithelial fragments were placed directly on the glass surface. After inserting them into culture tubes, 1.0 ml of nutrient medium was added.

Fibroblasts which had been maintained in continuous cultures, more than eighteen months, were prepared from a mother culture in a square bottle. One-ml quantities of cell suspension (approximately 3×10^4 cells) were transferred to each culture tube in which a coverslip had been inserted. The cultures were incubated at 37°C in a slanted stationary state.

The nutrient medium consisted of 80% Hanks BSS (containing 0.5% lactalbumin hydrolysate and 0.1% yeast extract) and 20% horse serum. Penicillin and streptomycin were added to it at a concentration of 500u/ml and 50 γ/ml respectively.

Herpes simplex virus of HF-HL_3 strain was maintained by serial passages in cultures of the strain of fibroblasts. When most of the cells inoculated with virus were affected and floated off into the medium, the fluid phase was centrifuged after three times of freezing and thawing, and the supernatant was harvested as virus seed.

Both epithelial and endothelial outgrowth began within 24 hours and formed a cell sheet around the original explant approximately 5 to 6 mm in diameter on the 4th day, when virus was inoculated. The strain of fibroblasts were used also on the 4th day of cultivation. Stromal cells, however, required 7 to 10 days to make a sufficient sheet for

inoculation.

For inoculation, in each experiment, the nutrient medium of the culture was replaced with 0.9 ml of medium containing 5% horse serum and this was followed by the addition of 0.1 ml of appropriately diluted virus suspension. To control groups medium was added instead of virus.

Those cultures were placed at 37°C and examined microscopically at intervals in the living states. At the appropriate time coverslips were removed, washed in BSS, fixed in Bouin's fixative and stained with Harris hematoxylin and eosin.

Results

Control groups.

Both stromal and endothelial cells showed so called fibroblastic growth and it was difficult to identify those cells by their morphology, except the fact that the latter sometimes arrange in a closely packed cell sheet similar to the epithelial one (Fig. 1,2,5,6). The epithelial cell was polygonal shaped and contained a round nucleus (Fig. 9, 10).

Infected groups.

The early sign of infection examined in the wet state was the appearance of round refractile or large swollen cells at the peripheral zone of the cell sheet. In epithelial cultures many huge cells which had protoplasmic bridges between them were observed.

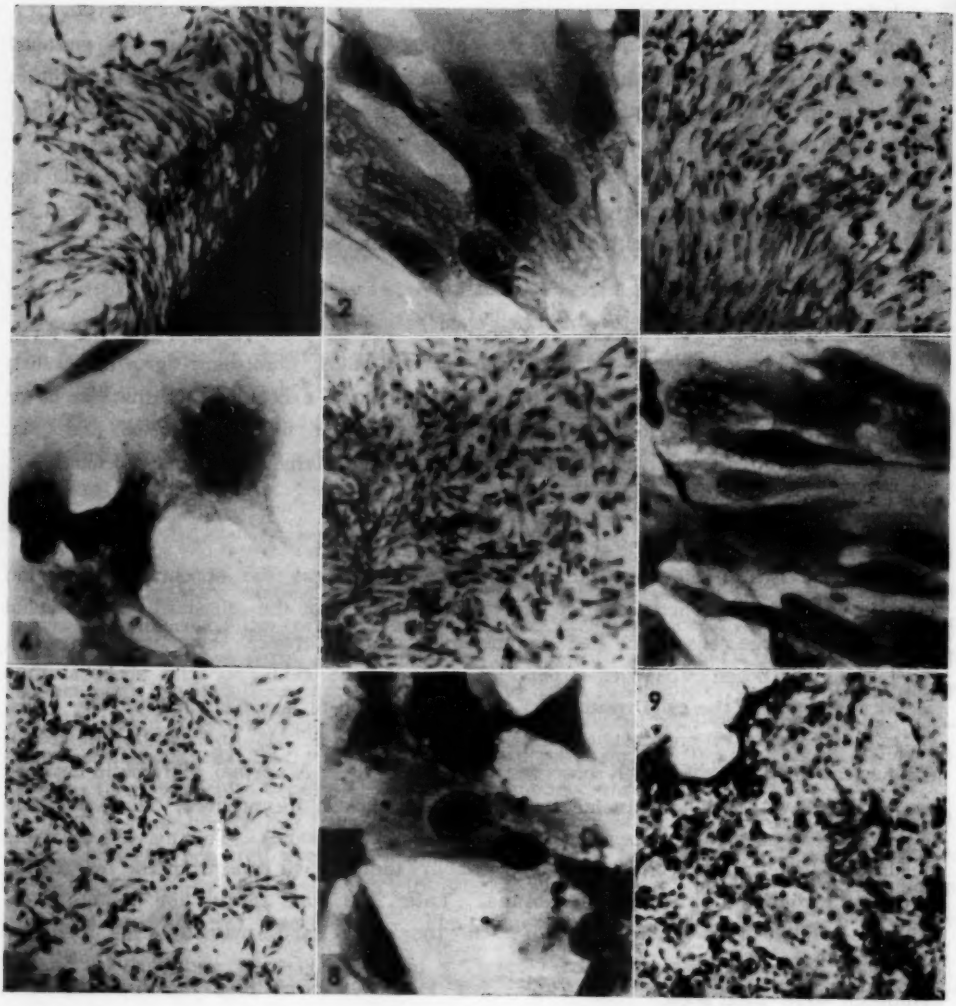
The cytopathogenic changes progressed towards the center of the sheet as time passed and later all the cells resulted in degeneration and at last sloughed off the glass.

In stained preparations destruction of the sheet (Fig. 3,7,11) and various stages of intranuclear changes were easily observed (Fig. 4,8,12,13). A normal chromatin network was replaced with finely granular substance and nucleoli disappeared, then purplish-pink homogeneous masses appeared among the chromatin strands. The masses coalesced to form a large mass which was separated by the rarefied zone from the marginated chromatin around the nuclear membrane. Later, the mass shrank and became acidophilic to form the type A inclusion body. On the other hand, pyknotic cells increased in number as the changes proceeded. Rounding of the cell shape and vacuoles in the cytoplasm were often seen.

The cytopathogenic effect was detectable in a stained preparation earlier than in an unstained one, and intranuclear changes were recognizable prior to the occurrence of alterations in the cytoplasm. This tendency was more evident in cells of the fibroblastic type.

Intranuclear changes mentioned above were not found in control cultures, although a few pyknotic cells were often observed.

In order to compare the susceptibility of the cells, stained preparations were examined and the extent of damage was recorded according to the following arbitrary standard scale.



Figs. 1 to 13. Preparations from experiment No. 4. Hematoxylin and eosin.

1. Normal endothelial cells. The darker area is the original explant. ×62.
2. Normal endothelial cells. ×620.
3. Endothelial cells 24 hours after inoculation with herpes virus. ×62.
4. Endothelial cells 24 hours after inoculation. ×620.
5. Normal stromal cells. ×62.
6. Normal stromal cells. ×620.
7. Stromal cells 24 hours after inoculation. ×62.
8. Stromal cells 24 hours after inoculation. ×620.
9. Normal epithelial cells. ×62.

0 =No change.

1+=Some of the cells showed intranuclear change.

2+=Half of the cells showed intranuclear change.

3+=Most of the cells showed intranuclear change.

4+=Sloughing of cells from the glass is remarkable.

The results of four experiments are summarized in Table 1. At each time interval, one or two cultures of each group were stained for examination.

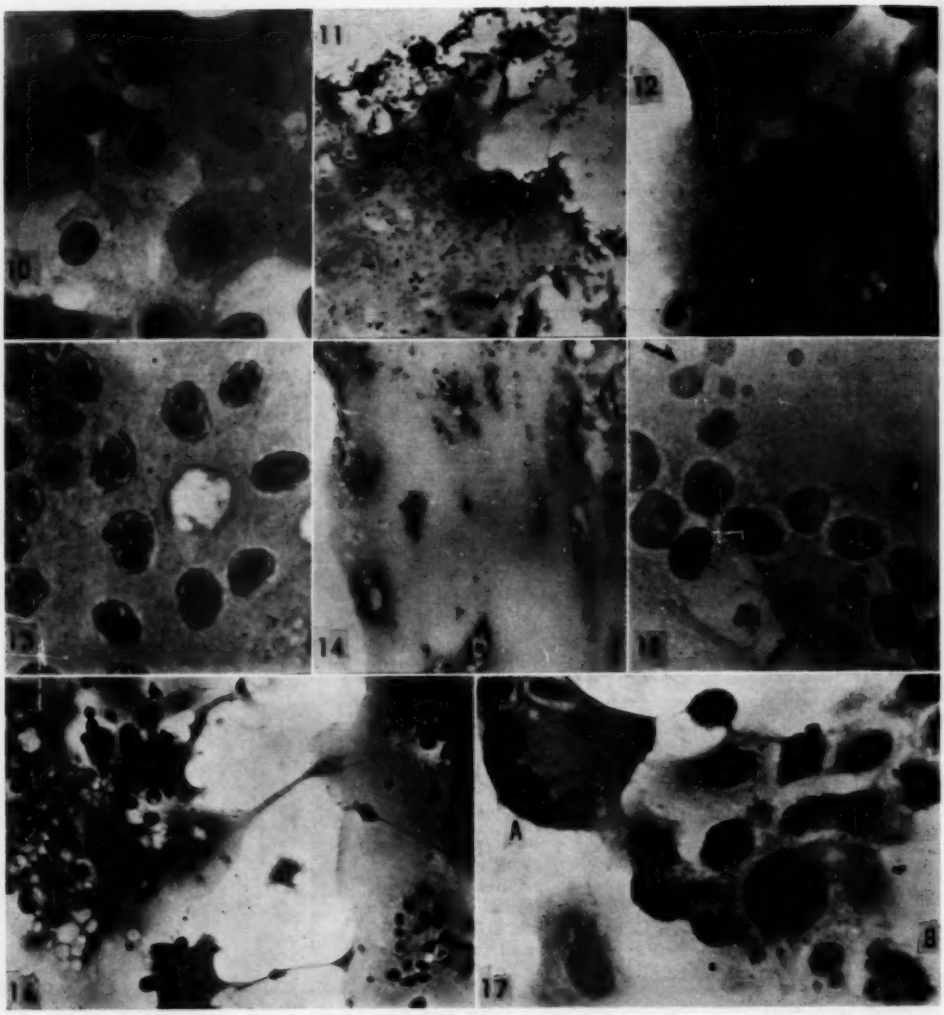
Table 1. Effect of Herpes Simplex Virus on Rabbit Corneal Cells at Different Hours after Infection

Experiment No.	hours / cell	6	12	18	24	36	48	72
1	Ep				3+			
	En				1+			
	St				1+			
2	Ep				3+		4+	4+
	St				1+		3+	4+
	F				1+		2+	4+
3	Ep	0	0	0	1+		3+	4+
	F	0	0	0	0		1+	1+
4	Ep	1+	2+	3+	4+	4+	4+	
	En	0	0	1+	1+	2+	2+	
	St	0	1+	1+	2+	3+	3+	
	F	0	1+	2+	3+	3+	4+	

Ep .....Epithelial cells.
En .....Endothelial cells.
St .....Stromal cells.
F .....Strain of fibroblasts.

Cultures showing poor growth were not used, so only in Experiment No. 4 all types of cells were available. When epithelial cells and fibroblasts were enumerated on the 4th day, the number of cell nuclei was 16×10^4 and 13×10^4 per tube respectively. Of course, adjusting the number of the cells in these primary cultures was difficult, but it was found that slight variation in the size of the cell sheet did not influence the result of each group. Epithelial cultures without plasma provided clearly stained preparations and appeared to show no significant difference from plasmatic cultures in the reaction to virus.

It is noticeable that cytopathic changes appeared earlier and proceeded more rapidly in epithelial cultures than in other ones. The stromal cells and the strain of fibroblasts were similar and resembled endothelial cells in their susceptibility.



10. Normal epithelial cells. ×620.
11. Epithelial cells 12 hours after inoculation. ×62.
12. Epithelial cells 12 hours after inoculation. Peripheral part of Fig. 11.×620.
13. Central part of Fig. 11. ×620.

Figs. 14 to 16. Preparations from experiment. No. 3.

14. Epithelial cells 24 hours after inoculaton. ×62.
15. Epithelial cells 24 hours after inoculation. Amitotic division (arrows). ×620.
16. Epithelial cells 48 hours after inoculation. ×155.

Fig. 17. The strain of fibroblasts 24 hours after inoculation.
Giant cells containing usual (A) and minute nuclei (B). ×620.

When higher concentrations of virus were used the changes progressed quickly.

Although the intranuclear change in an individual nucleus of those cell lines was similar, the process of formation of giant cells in epithelial cells was different from that of the others. In epithelial cultures the earliest sign of infection was dissolution of the boundaries of many cells, and nuclei appeared to collect together in aggregations (Fig. 14). Most of the nuclei showed early intranuclear changes and some of them exhibited amitotic division (Fig. 15). Those foci of infection were observed among normal cells, more frequently in the peripheral zone of the sheet. Later, these areas enlarged and became separated from each other by the lytic process of the cytoplasm but attached by protoplasmic strands (Fig. 16). In the early experiments, it was thought that this phenomenon was due to lysis of a plasmal clot, but it was observed also in cultures without plasma. Still later, as the intervening areas became lysed, there appeared large giant cells containing many nuclei, sometimes 100 or more in number, which had intranuclear inclusions in various stages. Small giant cells containing 10 or less nuclei were seen scattered among large giant cells. In several instances vacuoles were present in the cytoplasm.

In contrast, giant cells found in endothelial and stromal cells and the strain of fibroblasts were of the small type. Cells containing more than 10 nuclei, if ever, were very rare, and the process of their formation could not be clarified.

The nuclei in giant cells showed many varieties in their size, and some consisted of minute nuclei in which small inclusions were also observed (Fig. 17). In both types of giant cell, nuclei were likely to arrange around a central pink stained homogeneous area of the cytoplasm.

Comment and Conclusions

Herpes simplex virus has been regarded as epithelio-tropic, but in tissue culture it is known, that it shows a cytopathogenic effect on fibroblasts derived from many kinds of sources[1,4,8,12].

The cultivation of the separated layers of a corneal tissue that is epithelium, stroma and endothelium has been succeeded by several investigators[5,6,11,13].

In the present study, all kinds of primary cultures of rabbit corneal tissues exhibited the cytopathology specific to this virus and changes of individual nuclei seemed to be similar in those cell types. Details of the intranuclear changes were similar to those described by Scott et al[10].

When an equal quantity of virus was inoculated, changes in epithelial cells occurred earlier and progressed more rapidly than in the other three types of cells, that is stromal and endothelial cells and the strain of fibroblasts.

This result in tissue culture appeared to present information concerning the high degree of affinity of the herpes virus to the corneal epithelium in vivo. Any significant difference in the response to virus could not be detected between stromal cells and

the strain of fibroblasts. This observation seems to suggest that corneal fibroblasts have not changed their susceptibility to virus after prolonged cultivations. Endothelial cells resembled stromal cells in morphological changes and in susceptibility.

Gray et al[3]. succeeded in isolation of three different populations of herpes virus particles one of which, inoculated to HeLa cells, produced syncytial giant cells, a second of which caused a piling up of cells without large giant cells, and a third of which produced a rounding of cells without apparent piling up.

In the present study, the formation of large syncytial giant cells was observed only in epithelial cells and dissolution of the cellular wall, collection of many cell nuclei and separation of each large giant cell by the process of lysis were similar to the findings which Scott and his co-workers described as the giant cell response[3,9].

The cells of the other three types produced a rounding of cells and small giant cells without piling up and syncytial giant cell, the response called non-proliferative type.

Although the viral strain employed here might have contained different types of infecting agent, it appears that the cellular response to the virus varies according to the type of host cell.

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ON OVERACTION OF THE SUPERIOR OBLIQUE MUSCLE

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Overaction of the superior oblique muscle may be regarded as a syndrome inasmuch as various causes may be assumed. In this article, a critical analysis of 59 consecutive cases was made in order to elucidate clinical characteristics and causes of the overaction of the superior oblique muscle and evaluate surgical results by tenectomy of the muscle in question.

Incidence

The incidence of incomitence in squint differs according to workers. Duane stated 36%; White and Brown 34%; Bielschowsky 45% and Anderson 61%. In Japan Nakagawa found 32% among 458 cases.

It is conjectured that the discrepancies may be due to differences in criteria of diagnosis rather than racial or geographical difference. Kato[6] noted that true concomitance was a mere 10.7% when measured exactly. In fact, all degrees of incomitance may range from evident palsies to minor anomalies.

Vertical deviations are generally considered to be caused by defects in vertically acting muscles. Duane (1912) found the following defects in congenital ocular palsies: sup. rectus 54%, inf. rectus 33%, sup. oblique 7%, inf. oblique 6% (cit. by Berke[1]). Lyle[7] found, among 135 patients with vertical defects, the following figures: sup. rectus 59, sup. oblique 39, inf. oblique 6 and inf. rectus 11. Bielschowsky, however, insisted that weakness of the superior oblique muscle was most frequent. Many other different views were presented but the problem is still in controversy, presumably owing to difficulties in diagnosis and patterns of patients in question.

As a clinical picture, overaction of the inferior oblique muscle has its most common occurence among incomitant sqints, irrespective of which muscle is originally affected. Toselli[11] found the same 74% of incomitant cases.

In regard to overaction of the superior oblique muscle, Berke[1] remarked that it should not be uncommon and that it amounted to 39% vying in frequency with overaction of the inferior oblique (61%) when referred to Duane's figures.

The author[10] found in 182 incomitant squints that the occurrence of overaction of the inferior oblique muscle was 33% (61) and that of the superior oblique 32% (59). One of important reasons for such an unusual large incidence of the latter would be due to a characteristic pattern of squint in Japan. In western countries, esotropia is more frequent while, in contrast, exotropia is more common in our country. Crone[2] states that the ratio of esotropia was 96 : 4 in the Amsterdam youth and Nakagawa

and Suzuki[9] found that it was about 1:3 among school children in Sapporo. As regards clinical patients the ratio is about 1:1 in Japan. Thus it may be said that it would not be extraordinary that overaction of the superior oblique muscle is prevalent in this country as it is frequently associated with exotropia.

Case Analysis

Clinical manifestation of the overaction of the superior oblique muscle may be classified into two groups according to Nakagawa[8].

a. Simple Overaction (Type IV) .....36 cases

b. Overaction Associated with Underaction of the Inferior Oblique Muscle (Type V) .....23 cases

The incidence of both types is about 3:2, the isolated overaction being rather predominant. The secondary squint, which is defined here as subsequent to loss of vision of an eye, was found in 20% of the cases, almost all of which belonged to Type IV (Table 1). Clinically, the secondary squint at times displays incomitance, frequently being associated with overaction of the superior oblique muscle. This may be interesting in regard to its genesis.

Table 1. Incidence

	IV	V	Total
Primary	25 (70%)	22 (96%)	47 (80%)
Secondary	11 (30%)	1 (4%)	12 (20%)
Total	36 (100%)	23 (100%)	59 100%)

IV: SOOA. V: SOOA+IOUA

Table 2-(1). Age of Patient

	IV	V	Total
0			
1~ 5			
6~10	2	5	7
11~15	3 (1)*	2	5 (1)
16~20	12 (2)	2	14 (2)
21~25	7 (3)	8 (1)	15 (4)
26~30	4 (1)	1	5 (1)
31~35		1	1
36~40		2	2
41~	8 (4)	2	10 (4)
Total	36 (11)	23 (1)	59 (12)

* Secondary cases are shown in parenthesis.

The primary squint of Type IV had a relatively late onset as compared against Type V which has earlier onset (Table 2-(2)). Most of the former may be regarded as acquired and the latter congenital. Those of the earliest onset either in Type IV and V usually had

Table 2-(2). Age of Onset

	IV	V	Total
0	6	17	23
1~ 5	17 (5)*	3 (1)	20 (6)
6~10	4 (1)	1	5 (1)
11~15	4 (1)		4 (1)
16~20	1 (1)	1	2 (1)
21~25	1 (1)	1	2 (1)
26~30			
31~35			
36~40	1 (1)		1 (1)
41~	2 (1)		2 (1)
Total	36 (11)	23 (1)	59 (12)

* Secondary cases are shown in parenthesis.

Table 3. Vision

	IV	V	Total	%
1.0~2.0	9	4	13	46
0.6~0.9	11	3	14	
0.2~0.5	1	4 (A~ 2)	5 (A~ 1)	8
0.02~0.15	4 (*S~ 1, *A~ 2)	6 (A~ 6)	10 (S~ 1, A~ 8)	46
0~0.01	11 (S~10, A~ 1)	6 (S~ 1, A~ 5)	17 (S~11, A~ 6)	
Total	36 (S~11, A~ 3)	23 (S~ 1, A~12)	59 (S~12, A~15)	100

* S: Secondary cases. * A: With amblyopia.

Table 4. Binocular Vision

	IV	V	Total
S P (—)	9 (36%)	9 (45%)	18 (40%)
S P (+)	10 (40%)	11 (55%)	21 (46%)
F II	4 (16%)	0	4 (9%)
F III	2 (8 %)	0	2 (5%)

Table 5. Type of Squint

	IV	V	Total	%
X T	15 (S~ 2, A~ 3)	2 (A~ 2)	17 (S~ 2, A~ 5)	46
X Hypo T	5 (S~ 5)	5 (S~ 1, A~ 2)	10 (S~ 6, A~ 2)	
E T	10 (S~ 3)	11 (A~ 5)	21 (S~ 3, A~ 5)	46
E Hypo T	1	4 (A~ 2)	5 (A~ 2)	
E H T	1		1	
Hypo T	1		1	3
D H	1		1	
Ortho-phoria	2 (S~ 1)	1 (A~ 1)	3 (S~ 1, A~ 1)	5
Total	36 (S~11, A~ 3)	23 (S~ 1, A~12)	59 (S~12, A~15)	100

	IV	V	Total
X	20 (62%) (S~ 7, A~ 3)	7 (32%) (S~ 1, A~ 4)	27 (50%) (S~ 8, A~ 7)
E	12 (38%) (S~ 3)	15 (68%) (A~ 7)	27 (50%) (S~ 3, A~ 7)
Total	32 (100%) (S~10, A~ 3)	22 (100%) (S~ 1, A~11)	54 (100%) (S~11, A~14)

bilateral involvement of the oblique muscles with alternating fixation, unless one eye had poor vision. In contrast, those of later onset in IV had commonly uniocular fixation even when both eyes had good vision, with unilateral involvement in the deviating eye. The secondary squint always had uniocular overaction in the affected side. Bilateral involvement was found in 36% in Type IV and in 56% in Type V. As a rule, it may be said that the alternator has a bilateral involvement and the monocular deviator an unilateral involvement in the deviating eye.

Overaction of the superior oblique muscle was not encountered in early infancy but during adolescence or later (Table 2-(1)). Thus, the majority of cases were long-standing squints, in which overaction of the superior oblique muscle had gradually developed.

Vision in the non-fixing eye (poorer vision) is presented in Table 3. About half of the cases (46%) had relatively good vision and the remaining half (46%) disturbed vision. Comparing both types, IV had somewhat better vision than Type V. The cause of visual debility was due to corneal opacity, retinal diseases etc., in Type IV and almost exclusively amblyopia ex anopsia in Type V. Loss of vision in later life usually accompanies exotropia, which is sometimes associated with the simple overaction of the superior oblique muscle. Exodeviation may help, as Jampolsky[5] stated, for developing overaction of the superior oblique but it is not the necessary condition. To demonstrate such instances, Case 1 and 2 are presented,

where the simple overaction developed without exodeviation.

In the great majority of cases binocular vision was deeply disturbed as shown in Table 4. Only six cases in Type IV had fusion with amplitude (F II) or stereopsis (F III). After surgery with or without orthoptics, binocular vision was more or less improved in about half of the cases. An anomalous correspondence was found in 41% by after-image test.

Overaction of the superior oblique muscle was usually associated with horizontal deviation (Table 5). Exotropia was predominant in Type IV and esotropia in Type V. In the secondary squint, the exotropia : esotropia ratio was 8 : 3. In the primary squint in Type IV it was 13 : 8. In Type V, the primary squint without amblyopia was usually associated with alternating esotropia and that with amblyopia was associated with an equal number of esotropia and exotropia. Generally speaking, exotropia was prevalent in the acquired squint group and esotropia in the congenital group.

Vertical deviation (defined as greater than 5° in the primary position) was rather rarely found. When it existed, it was usually slight. There were only 3 cases in which their vertical deviation surpassed 10°. It may be considered that the oblique muscle anomalies chiefly affect the oblique position of the eye (the tertiary position) and does not have such an extensive effect on the primary position as compared with that of the vertical rectus anomalies.

The grade of the oblique muscle anomalies may be properly expressed in terms of the "corner" deviation, i.e. vertical deviation in the maximum field of action of a muscle. In overaction of the superior oblique muscle, it varied from 5° to 30°, being 10° to 15° in most cases. Underaction of the inferior oblique muscle had rather minor degrees, varying from 5° to 10°. In a few instances, however, the underaction was much greater than its accompanying overaction.

The A syndrome, as Berke remarked, is a common complication. It was found in 36% of Type IV and in 74% of Type V, the average being 51%. Overaction of the inferior oblique muscle is usually associated with the V syndrome, the percentage of which is less than that of the A syndrome in the presence of overaction of the superior oblique[8]. It is evident that the A-V syndrome has a close bearing on the oblique muscle anomalies. Complication of the A syndrome was 38% in case of unilateral involvement and 68% in case of bilateral involvement in the average of both types. The A syndrome may be caused by the increased abduction in downward gaze by the overacting superior oblique muscle. It may be cured by a suitable correction by tenectomy of that muscle.

The chief complaints of the present series were as follows: squint operation 47%, visual debility 15%, tatooing 10%, diplopia 5%, asthernopia 5% etc.

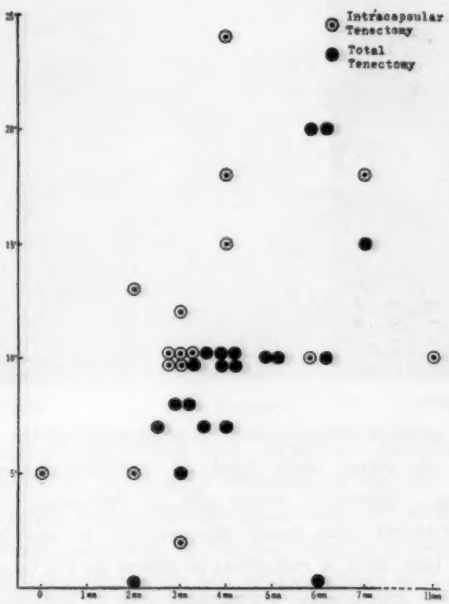
Ocular torticollis was found in 42% of the cases, especially in congenital squint but, except in a few instances, it was not characteristic and was of no aid for the diagnosis of the affected muscle.

Surgery

Berke's intrasheath tenectomy of the superior oblique muscle is an effective and efficient procedure. However, regardless of his warning against severing the sheath, total tenectomy (tendon together with its sheath) advocated by Nakagawa is safe and equally efficient.

The results of surgery are presented in terms of the corner deviation (Fig. 1). The effect, though rather fluctuating, was proportional to the amount of tenectomy, the average being 2.5° per 1 mm. For 10° of corner deviation, a 3-4° mm. tenectomy is suitable. No difference in the effects was seen between the intrasheath and total tenectomies. The maximum correction attained was 20° with a 6-7 mm. tenectomy. Overcorrection or paresis of the superior oblique muscle never occurred within the author's experience. When undercorrected, resection of the contralateral inferior rectus muscle was further indicated. In cases associated with underaction of the inferior oblique muscle, when necessary, a strengthening operation (tucking or resection) was conducted on the muscle. In most cases tenectomy of the superior oblique could correct, eventually, underaction of its antagonist when it was slight. The effect of the tenectomy was usually long-lasting and the strengthening operations on the inferior oblique muscle were proved insufficient and temporary.

Fig. 1. The effect by the tenectomy of S.O.



Tenectomy of the superior oblique caused almost no change in horizontal deviation and even in the presence of slight changes, a few degrees of esodeviation resulted at the most.

Following tenectomy, at times a temporary excyclophoria (approx. 10°) resulted and patients complained of tilting images. However, a permanent annoyance due to tenectomy never occurred even when bilateral tenectomies were made.

Surgery was carried out in 29 patients in whom 36 eyes were tenotomized. A 78% improvement (the residual corner deviation is less than 4°.) was seen and, when the initial deviation was less than 10°, a 90% improvement was seen. The final results of surgery of 29 cases were as follows:

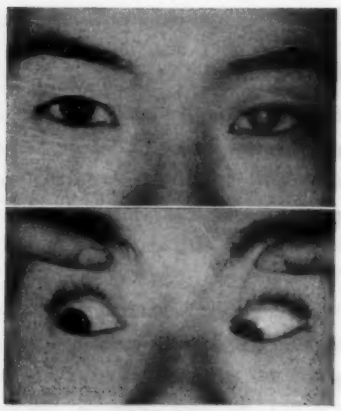
a. functional cure (fusion or stereopsis 11 (38%)
b. cosmetic cure (concomitant with no fusion) 9 (31%)
c. imperfect cure 9 (31%)…total 29 cases.

Case Reports

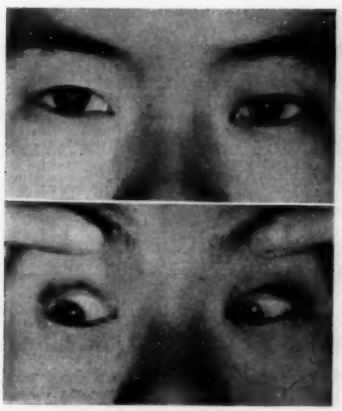
Four cases are presented here by way of illustration.

Case 1. is a simple overaction of sup. oblique due to loss of vision (leucoma adherens 10 years ago). There was no exotropia in this case. The downward overshoot of the left eye (ca. 8°) was brought be normal by a 3 mm. tenectomy of the left sup. oblique. The primary position of the eyes was not affected by the oblique muscle surgery.

Case 1.



Before Surgery



After Surgery

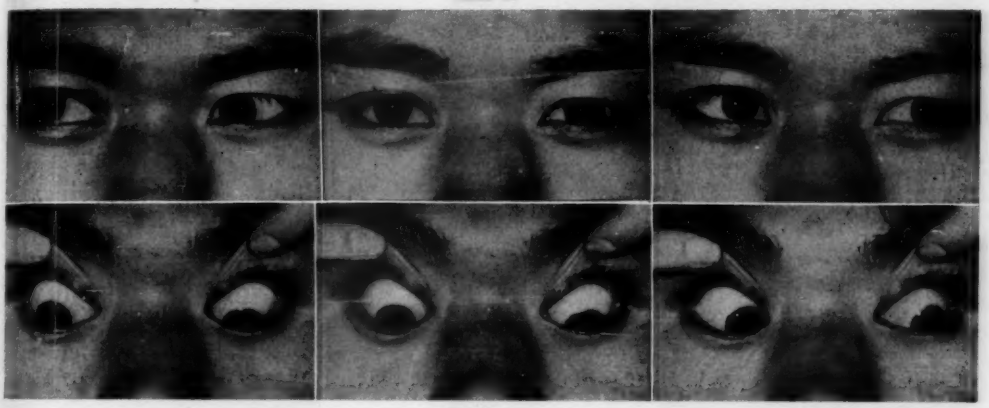
Case 2. indicates a gradual development of overaction of the superior oblique muscle due to reduced vision. A young male aged 24, complained of ever increasing diplopia of 3 to 4 years standing. About 9 years ago, a diathermy operation on the same patient for retinal detachment was made successfully in our clinic in the upper-inner quadrant of the left eye ball, with a reduction of vision to 0.1 (n.c.). There was a slight apparent underaction of the inf. oblique presumably due to the adhesion of fascia to the bulbus.

In eyes straight no deviation or diplopia was seen. Only in eyes right diplopia was noticeable, which increased in downward gaze. In this direction the left eye overshooted

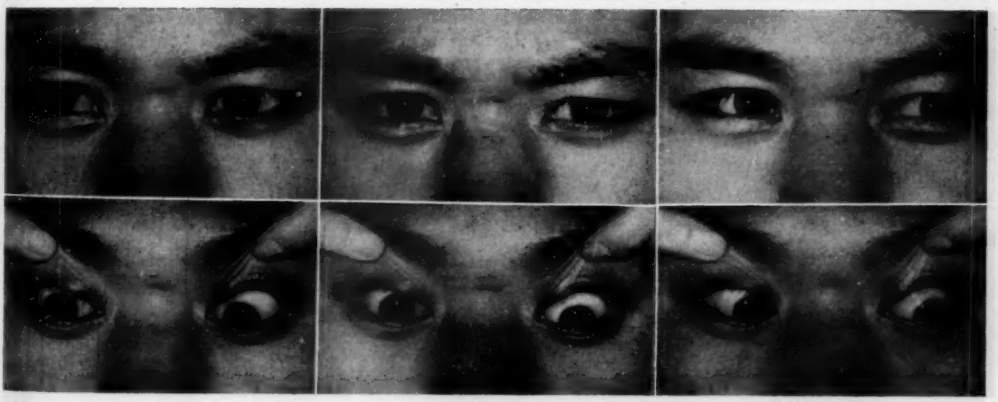
downward ca. 15° and exodeviated ca. 10°—the A syndrome.

The condition was corrected by surgery: a 11 mm. intrasheath tenectomy of the left sup. oblique, supplemented by a resection of the right inf. rectus. No change was produced in the primary position of the eyes by the operations.

Case 2.



Before Surgery

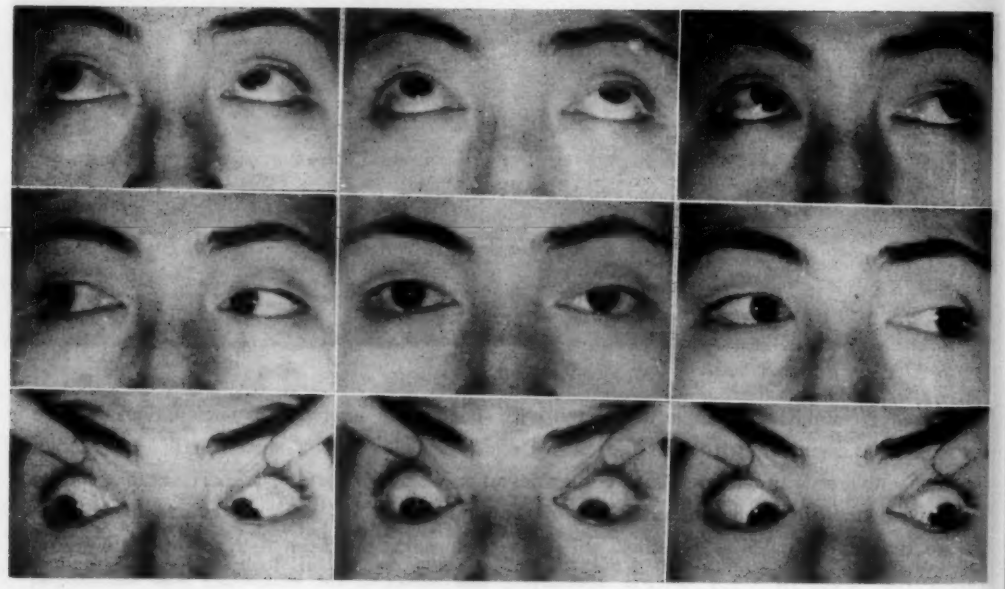


After Surgery

Case 3: simple overaction associated with congenital exotropia. The patient was a young male, aged 20, with an alternating exotropia of ca. 35° since birth. Overaction of sup. oblique was bilateral, each corner deviation being 10° after the horizontal deviation was corrected. By a 3mm. tenectomy of the sup. oblique in each eye ocular movements were reduced to normal together with the disappearance of the A syndrome and fusion improved from SP (—) to FII thereby. The effect of the tenectomy was unchanged in an 8 year follow up.

Case 3.

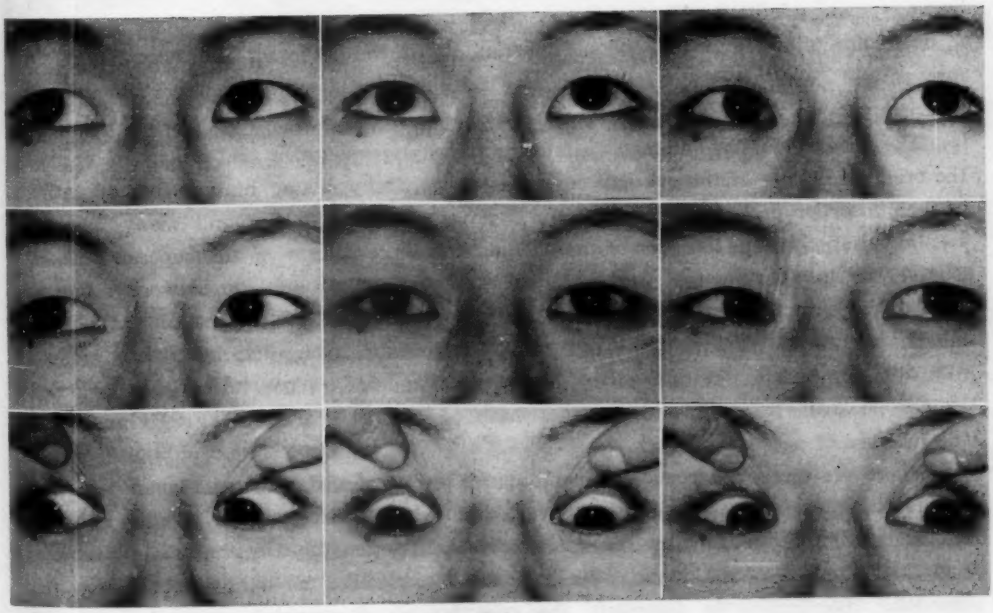
Before Surgery



After Surgery

Case 4.

Before Surgery



After Surgery

Case 4: overaction of the sup. oblique associated with underaction of the inf. oblique (Type V). The patient was a young male, 22, with an alternating esotropia since birth. There was no appreciable vertical deviation in the primary position. Overaction of sup. oblique in terms of corner deviation was as follows: R 8°; L 13° and underaction of inf. oblique: R 5°; L 5°. Esotropia increased in eyes up (E 15°) and decreased in eyes down (X6°). Surgery was performed only in the left eye; a 2 mm. intrasheath tenectomy with a 5 mm. recession of med. rectus. Overaction of sup. oblique in the opposite eye (right) was increased temporarily after the operation and decreased with lapse of time.

The photograph presented was taken 4 years after the surgery, in which the ocular motility was almost concomitant except in left-upward gaze where underaction of the right inf. oblique remained unchanged.

Discussion

The cases of vertical deviation associated with horizontal strabismus are obscure and are subject to speculation at the present stage of our knowledge. It may be produced by various causes, such as palsy of vertically acting muscles, their congenital maldevelopment, anomalies in the fascia bulbi or sites adjacent to these muscles etc.

Overaction of the superior oblique muscle, according to orthodox consideration, is paretic in nature. It may be caused by palsy of the ipsilateral inferior oblique muscle or the contralateral inferior rectus muscle. The isolated palsies of these muscles, however, are admitted to be very rare. It is difficult to interpret common ocurrence of overaction of the superior oblique muscle. It follows that with the exception of a few paretic cases, therefore, the majority of the present series would be nonparetic in origin.

The oblique muscles differenciate latest in the phylogenetic scale (in the stage of bipeds), the original rotators of the eye ball developing to vertically acting muscles by migration of their insertion and of the origin of the superior oblique. Fink[4] found many developmental variations in the insertion of the oblique muscles, especially of the superior oblique. Duke-Elder[3] pointed out that congenital defects in the motility of the eyes were common. It might be expected that an anomalous motility due to maldevelopment of the oblique muscles occurs not infrequently. As fusion is consolidated, congenital defects are reduced to a minimum and the motility of eye becomes concomitant. When fusion is undeveloped or lost, however, it remains or becomes manifest and will even be exaggerated with the increase of age.

There are three antagonistic systems which are concerned with tonic balance of the extraocular muscles, i.e., the horizontal recti, the vertical recti and the oblique muscles. In the horizontal direction the internal rectus muscle usually overacts in early infancy and the external rectus muscle becomes more overactive in later life. When the binocular coordination is disturbed, the eyes become esotropic in early infancy and

exotropic later. As the horizontal muscles are more flexible, the horizontal deviation is commonest in squint. On the contrary, the vertical balance is rigid and most stable, presumably due to the "frame work" of the fascia bulbi (Fink), and vertical deviation due to vertical rectus anomalies is very rare. In a few cases in the secondary exotropia, however, the overactive superior rectus was encountered, sometimes being associated with the overaction of the superior oblique.

The pair of the oblique muscles are concerned, as the primary action, with the oblique position of the eyes as already shown. The superior oblique muscle acts as a depressor when the eye is adducted or converged and plays an important role in the reading position of the eyes. In infancy, overaction of the inferior oblique muscle has its commonest occurrence in the oblique muscle anomalies and decreases or is reduced to normal with increasing of age, as it is rarely found in adults. On the other hand, the superior oblique muscle becomes overactive in later life as shown in Type IV. As Jampolsky pointed out, exodeviation would help development of this condition. Congenital underaction of the inferior oblique (usually bilateral) is frequently associated with overaction of the superior oblique which is more pronounced with the increase of age. This type of the oblique muscle anomaly is commonly found in congenital alternating esotropia (Type V).

Congenital oblique muscle anomalies are usually bilaterally involved and may be classified into two groups, i.e., overaction of the inferior oblique muscle associated with the underaction of the superior oblique and overaction of the superior oblique associated with underaction of the inferior oblique. They are frequently found in esotropia as the action of the oblique muscle is accentuated in esodeviation. These two groups do not differ in nature but are graded variations in developmental anomalies. The "rest position" of the oblique muscle may be upward or downward as in the case of the horizontal squint in which the rest position is inward or outward according to the congenital muscular balance.

Conclusion

Critical analysis was made on 59 concecutive cases of overaction of the superior oblique muscle and the following results were obtained:

1. Overaction of the superior oblique is common in Japan, presumably due to the much greater incidence of exotropia than esotropia in this country. It was found in 32% of incomitant squint, being in the same order of magnitude with overaction of the inferior oblique in occurrence.

2. There are two types of overaction. The simple (isolated) overaction of the superior oblique is acquired in nature and found in the non-fixing eye, which developes gradually in a longstanding squint, usually associated with exotropia. The congenital overaction of the superior oblique overaction is usually accompanied by underaction of the inferior oblique, being involved bilaterally and frequently associated with esotropia.

3. In the great majority of cases, the cause of overaction of the superior oblique is assumed to be non-paretic in origin and presumably due to its developmental anomalies. The oblique muscles, superior and inferior, are antagonistic in tonus as well as in action, as in the case of the internal and external recti. As a rule, the inferior oblique is overactive in infancy and the superior oblique eventually overacts in later life. When binocular coordination is undeveloped or lost by some reason, the superior oblique muscle will become overactive.

4. The intrasheath (Berke) and total (Nakagawa) tenectomy of the superior oblique are equally efficient surgical procedures for the correction of overaction of that muscle. In average a 1 mm. resection of the tendon reduced 2.5° in the corner deviation. The results in cure were 78% in 36 eyes.

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RETINAL BLOOD FLOW

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It is a current problem in the medicine to study systematically the pathologic physiology of the circulation in various conditions of organs such as kidney, brain, heart, lung, liver, and so on. Many active investigations are being performed both in the clinical and basic field. The latest progress of the method of measurement affords a better understanding of the problem.

From early times, the tissues of eyeball, especially the retina is considered to be very important in its relation with brain or kidney. Accordingly, it should be very interesting, if the retinal blood flow could be measured and be compared with renal or cerebral blood flow.

We have been engaging in measurement of the caliber of retinal blood vessels and the blood pressure in the retina, for over ten years. The caliber of retinal blood vessels is measured by Mikuni's eye piece attached to the large simplified Gullstrand's Ophthalmoscope, and the retinal blood pressure by Mikuni's Ophthalmodynamometer.

Method

Hagen-Poisseuille's law is useful to find out the relation among blood flow, width of the blood vessel and defference of blood pressure for some distance of the blood vessel.

Although there might be something to be discussed on this method, still we are able to calculate the retinal blood flow (i) by the following formula.

$$i = \frac{\pi(\frac{a}{2})^4}{8\eta l}(P_0 - Pr)$$

a……Width of retinal artery.

l……length of the artery between the bifurcation point of the ophthalmic artery from the internal carotic artery and optic nerve head.

P_0……Blood pressure of internal carotic artery.

Pr……Retinal artinal blood pressure.

η……Viscosity of blood.

Blood pressure is represented by middle blood pressure according to Wezler & Böger's formula.

Middle blood pressure diastolic blood pressure. + (0.42+Pulse pressure value.)

Branchial blood pressure is placed equal to the internal carotic blood pressure.

1 is looked upon as constant. η is also regarded as constant when no change is observed in blood viscosity. Such constants can be placed as K.

$$\frac{\pi}{8\eta l} \cdot (\frac{1}{2}) = \text{CONSTANT} = K$$

$$i = K.a^4.\ (P_0 - Pn)$$

Blood flow in relative value. $= a^4\ (P_0 - Pn)$

The retinal blood flow is calculated very easily in relative value. The relative values are enough to compare the values of blood flow from one another.

The relative value multiplied by K turns into the absolute value.

If these formula are as follows:

l=3.7 cm……mean value of 9 cases in 5 males and 4 females, measured anatomically,

η=4.57 0.10087 g/cm. sec.……mean value of 10 cases measured by Hess's Viscosimeter,

then this K will be the following number. $K = \frac{19.4}{10}$

Results

First, the results seen in healthy persons are presented here. 73 persons; 37 males, 36 females, aged from 12 to 69 years as shown in table 1 provided the measurements. Values of blood flow calculated are shown in table 2.

Table 1. Number of healthy persons.

sex \ age	12~19	20~29	30~39	40~49	50~59	60~69	total
male	3	9	11	6	5	3	37
female	5	10	7	4	5	5	36
total	8	19	18	10	10	8	73

Table 2. Relative values in normal persons.

sex \ age	12~19	20~29	30~39	40~49	50~59	60~69	mean
male	70.82	75.22	80.78	73.81	73.65	61.09	76.44±8.74
female	71.84	69.03	55.61	68.54	58.75	68.80	69.51±7.92
mean	72.37±8.01			68.79±7.97			71.00±6.51
	72.25±7.64			67.18±9.53			

The value in the female is a little less than in the male, though we can find on significant difference between the two sexes. No. change is observed from persons of 12 to 49 years, but a significant decrease is found after 50 years.

The relative value multiplied by K turns into the absolute value. The absolute value of blood flow in arteria temporalis inferior or superior are shown in table 3.

Next the results for 80 cases with essential hypertension are presented.

Keith-Wagener's criteria is employed for the classification of the stages in essential hypertension. Percentage changes of retinal blood flow in the stages of hypertension, with the value in normal average retinal blood flow is placed at 100%, are shown in table 4.

Table 3. Absolute values in healthy persons.

sex	No. of cases	absolute value (cc/sec)
male	37	0.00148±0.000169
female	36	0.00135±0.000153
mean	73	0.00138±0.000126

Table 4. Blood flow (%) in essential hypertension.

K.-W.	Ne.of cases	mean
I	35	87.74±10.24
II	36	68.01±11.64
III	5	38.79±25.20
IV	4	34.53±22.81

Various degrees of decrease were observed; 87.7% in the cases of K.-W.'s group I, 68.0% in K.-W. II, 38.8% in K.-W. III, and 34.5% in K.-W. IV.

A significant decrease was found with the progress of the stages in the hypertension and most remarkable in K-W III and IV.

Various degrees of the percentage decrease in retinal blood flow were found in the retinopathies as seen in table 5. The percentage value in 10 cases with renal retinopathy is 34.6%, in 3 cases with retinopathy in late toxemia pregnancy 35.1%, in 6 cases with arteriosclerotic retinopathy 64.3%, and in 5 cases with diabetic retinopathy 86.4%.

Both the decrease in renal retinopathy and retinopathy of late toxemia pregnancy was approximately equal to K.-W. III. The decrease in diabetic retinopathy to K.-W. I and arteriosclerotic retinopathy to K.-W. II.

The percentage values of retinal blood flow in 13 patients obtained before apoplectic fit are shown in table 6. The values are 43.2% in 2 cases of K.-W. I, 43.2% in 6 cases of K.-W. II, 33.0% in 1 cases of K.-W. III, and 26.4% in 4 cases of K.-W. IV.

Table 5. Blood flow (%) in retinopathies.

retinopathies	No. of cases	mean
renal	10	34.60± 8.13
late toxemia pregnancy	3	35.19± 7.96
arteriosclerotic	6	64.33±13.96
diabetic	5	86.48±16.03

Table 6. Blood flow (%) in the cases with apoplexy.

K.-W.	No. of patients	mean
I	2	43.25
II	6	40.81
III	1	33.09
IV	4	26.65

All these values are very smally compared with each stage of hypertension without apoplectic fit as seen in table 7. The values in the cases of K.-W. I and II before apoplectic fit correspond with the cases of K.-W. III or IV without apoplexy.

Table 7. Comparison between cases without and before apoplexy fit.

essential hypertension without apoplexy			hypertension before apoplexy fit		
K.-W.	No. of patients	mean	mean	No. of patients	K.-W.
I	35	87.74	43.25	2	I
II	36	68.01	40.81	6	II
III	5	38.79	33.09	1	III
IV	4	34.53	26.65	4	IV

Comment

No literature can be found in reference to our results on retinal blood flow. However, our retinal blood flow is compared with renal blood flow and cerebral blood flow in the literature published in Japan. Renal blood flow published by Ogino (1958), Kyoto university, Department of Internal Medicine, makes a good comparison with our retinal blood flow as seen in fig. 1. Renal blood flow decreased with the progress of the stages in essential hypertension. We found a close parallel between renal and retinal blood flow, whereas there was no close parallel between cerebral and retinal blood flow. This depends on the method in measurement of blood flow, we think. Cerebral blood flow is quoted from the paper written by Aizawa (1956), Keio-University, Department of Internal Medicine. We can say the measuring method of cerebral blood flow is very complicated and not excellent at the present time.

Fig. 1

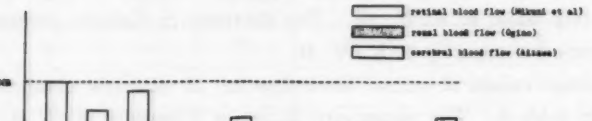
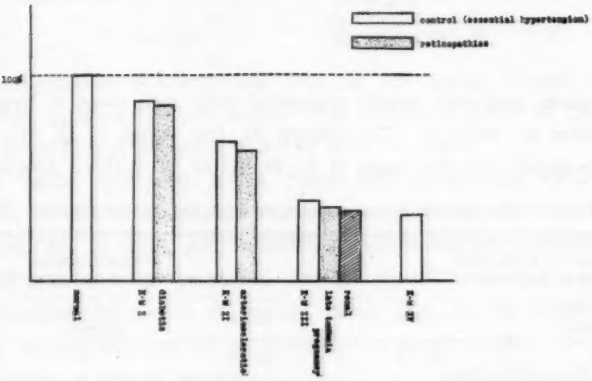


Fig. 2



The values of various sorts of retinopathies are shown compared with those of 4 groups of essential hypertension in figure 2. The value in renal retinopathy presents an almost complete similarity to that in retinopathy of late toxemia pregnacy and they are quite similar to that of K.-W. III, too. The similarities of the value in diabetic retinopathy to that of K.-W. I and the value in arteriosclerotic retinopathy to K.-W. II are noticeable.

Fig. 3

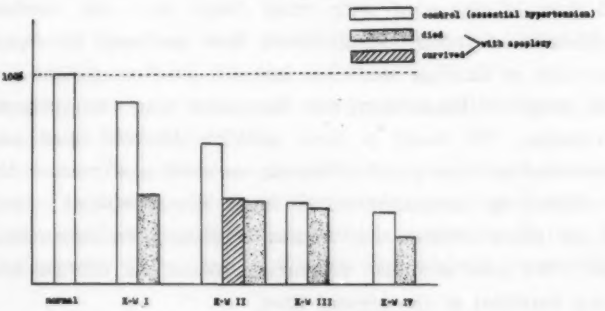


Fig. 3. establishes a comparison between patients of essential hypertension without apoplexy and with apoplexy before fit. Comparing the cases with and without apoplexy, a remarkable decrease of blood flow is observed in the former.

Especially in the cases of K.-W. I and II with apoplexy, the decrease is so evident that we can compare it favorably with patients of malignant hypertension (K.-W. III and IV) in the degrees of decrease. Accordingly, even if in a case of benign hypertension, it is considered to be in grave danger of apoplexy, when a remarkable decrease of the retinal blood flow comparable with that of malignant hypertension is noticed.

We can find many literatures referring to relations between cerebral circulation and apoplexy. Kety's N_2O method is usually employed to study cerebral circulation, however, Kety's method is very complicated to perform. We prefer our method of retinal blood flow to Kety's method in order to study retinal and therefore cerebral circulation. Our method of measurement of retinal blood flow is expected to be useful in daily practice. An exact finding can be obtained by this method and this method can be practiced easily, painlessly, and repeatedly, if necessary.

Conclusion

A method to demonstrate retinal blood flow was described. Blood flow is obtainable not only in relative but also in absolute value. Values in normal persons, in the cases with essential hypertension, in various kinds of retinopathies such as renal, late toxemia pregnancy, arteriosclerotic and diabetic retinopathy, and in the cases with essential hypertension before apoplectic fit were studied.

This method to demonstrate retinal blood flow is expected to be useful in daily practice. An exact result can be obtained by this method, and it can be practiced easily and painlessly and repeatedly if necessary.

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STUDIES ON ERG UNDER DEEP HYPOTHERMIC ANESTHESIA

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Hypothermic anesthesia brings about usually a drastic decline in metabolic rate. What effect has hypothermic anesthesia upon the function of the retina which generally has a higher metabolic rate? The answer made by means of the electroretinographic examination as to this question will be informed in this report.

Methods

Healthy adult mongrel dogs weighing about 10 kg were used for the experiment. Hypothermic anesthesia was performed, using ether, by a closed circulation technique with tracheal intubation. This technique was described in earlier papers[1,2,3] by Okamura and his coworkers. The animal enclosed by a vinyl sheet was cooled by immersing his whole body in an ice water bath. Rewarming was also performed by placing the animal body in a warm water bath of 47°C. The temperature of the animal body was measured with an alcohol thermometer inserted into the rectum.

Recordings of the ERGs were made with an ink-writing oscillograph in a large room shielded from the electric disturbances, under an uniform artificial illumination of 8 lux, provided by diffuse rays transmitted through an opal glass plate. The contact lens electrode of Riggs' type[4] was used as a pick-up electrode of ERG. A neutral electrode was attached on the frontal skin area above the examined eye.

The intense flash light of a gas discharge lampe was used as a stimulus whose intensity and duration were about 1,800 lumen per second and 0.66 millisecond respectively.

Results

The ERGs taken in the course of cooling by the ice water bath as well as in the course of rewarming by the warm water bath were presented in Fig. 1, each curve indicating changes in form of the ERG at each temperature of the body.

As can be seen in Fig. 1, both of the negative wave which appeared immediately after the light stimulus was given and of the subsequent positive wave showed gradual decrease in amplitude with decrease in body temperature, untill the ERG extinguished at 25°C. At the body temperature lower that 25°C no ERG could be detected at all.

After the body temperature during the cooling attained down to 22°C, the thoracotomy was performed at the left 5th intercostal region and all veins entering the heart were quickly obstructed by means of basal forceps. Then, the subsequent obstructions of both the pulmonary artery and the ascending aorta made by means of Kisser's ligation were followed almost simultaneously. And, 8 cc of 2.5% potassium

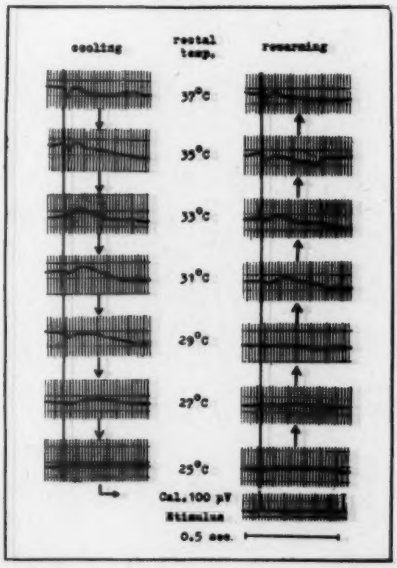
citrate solution was rapidly injected around the original part of the ascending aorta in order to cease the cardiac beat. By the injection of this solution, the cardiac beat was arrested in about 30 seconds after the beginning of the injection. Thereafter, the standstill of the heart beating and the cessation of the respiratory movement were maintained for 50 minutes. During this standstill period, the temperature of the animal body reached the minimum at 19°C.

When the arrest of the blood circulation was released, the heart massage was carried on for 8 minutes with a beat rate of 40 per minute. The heart beating was strengthened by an injection of 0.4 cc of noradrenaline into the left ventricle of the heart. At the same time, rewarming was started, being kept under the controlled respiration. As soon as the chest was closed, the movement of respiration reappeard spontaneously, but it was aided still by an auxiliary respiration, untill the body temperature arrived at above 30°C. When the body temperature restored fairely well to the normal level, the intratracheal tube was removed.

The animals were generally sacrificed in three months after the operation.

As presented in the rewarming course of the right series in Fig. 1, the ERG potential appeared, in a slightest degree, at first in the time when the body temperature rose to 27-29°C. With the subsequent gradual rise in body temperature, the heights of the negative and positive waves increased. These variations in ERG indicate the characteristics opposite to what were observed during the cooling course, as shown in the left series of Fig. 1.

Fig. 1



Summary

The relationship between the variation in amplitude of ERG and the changes in body temperature are presented in Fig. 2. The amplitudes of the negative and positive waves are plotted as ordinates against the body temperature as abscissae.

The data of various physiological conditions during the hypothermic anesthesia are graphically shown in Fig. 3.

Fig. 2

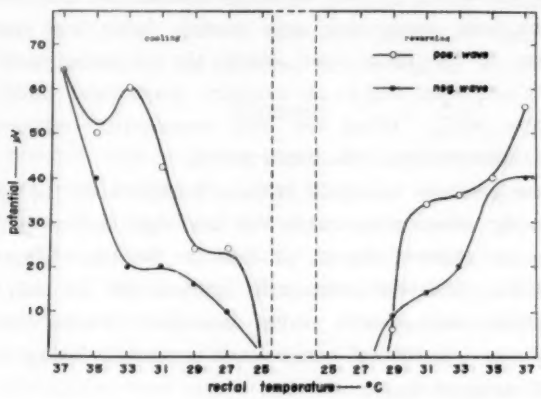
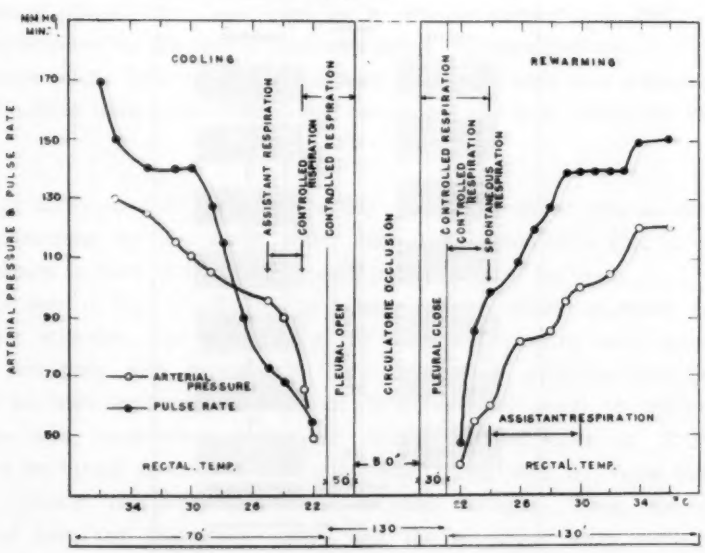
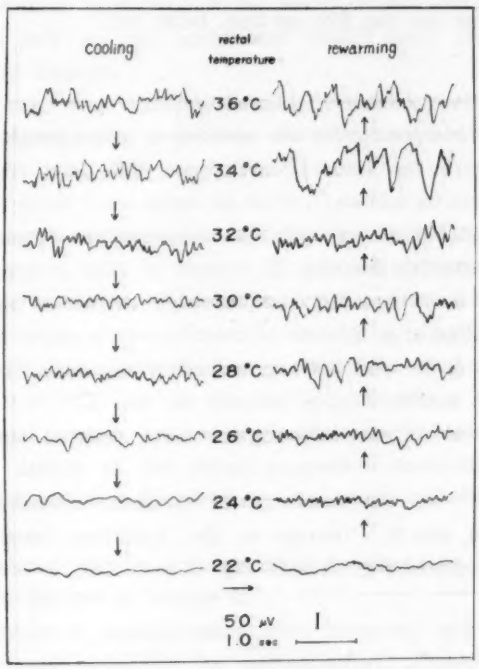


Fig. 3



In order to elucidate the effect of the hypothermic anesthesia on the central nervous system, electroencephalographic (EEG) studies were also made at the same time. The experiment was carried on under the controlled anesthesia, taking data of the following five measures: EEG, ECG, femoral arterial blood pressure, respiration rate and pH of the carotid arterial blood. Among them, some examples of the frontal EEG were illustrated in Fig. 4. PH of the carotid arterial blood was kept practically constant at 7.4 during the hypothermic anesthesia.

Fig. 4



Conclusions

Tissue metabolism in the dog retina was confirmed to decline electroretinographicaly with decrease in body temperature. The ERG extinguished perfectly within a range of the body temperature lower than 25°C. Rewarming makes the ERG to develop again and, when the body temperature restores perfectly to the normal level, the ERG shows again the normal feature.

Thus, the retinal tissue is capable to recover fully even from such a state of artificial hibernation, that the body temperature reached 19°C, and the blood circulation was arrested for 50 minutes.

It is concluded that hypothermia has an inhibitory effect on the development of ERG, and that this inhibitory effect disappears with restoration of the body temperture without remaining any damage of the retinal function.

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Correction

In the article "Mottled fundus in association with pseudoxanthoma elasticum" by Dr. K. Shimizu in the January-March issue, 1961, four errors were corrected. These sentences were as follows:

1. (Plate 1 & 2, Fig. 1) was left out following the term (Fig. 1 & colored plate: Figg. 1) in the 7th line of p. 2.

2. (Plates 3,4 & 5) was left out following the term (colored plate: Figg. 2,3 & 4) in the 6th line of p. 4.

3. (Plates 6,7 & 8) was left out following the term (colored plate: Figg. 5,6 & 7) in the 29th line of p. 5.

4. (Plate 9) was left out following the term (colored plate: Fig. 8) in the 7th line of p. 10.

In the article "On the reactivity of choroidal reticuloendothelial system" by Dr. A. Urayama, and N. Yamada in the April-June issue, 1961, the colored photographs were replaced Fig. 12 with Fig. 13 in p. 148.

Book Review

" Genetics and Ophthalmology. " Volume 1. By P.J. Waardenburg, A. Franceschetti, and D. Klein, Assen, Netherland, Royal Van Gorcum Ltd. 1961. 992 Pages, 797 illustrations, 53 in color. HFL. 135.00.

This book comprises the newest of genetic knowledge in chapter 1, there fore readers will be able to obtain the latest news of general genetice and human genetics.

A number of useful illustrations, valuabie pedigrees, phtographs and literatures are given in every chapter.

This book gives also a full explanation of the developmental and anatomical knowledge in every chapter, in this way we can understand easily many characteristic signs and symptoms of hereditary diseases.

In chapter one, this book deals with some elementary statistical and biometrical notions, indispensable for population genetics and the ascertainment of conclusions in general.

The contents of Volume 1 are given as under ;
genetics and the human eyes in chapter one, eyebrows and eyelide in chapter 11, affections of the conjunctiva palpebrae et bulbi in chapter III, lacrimal apparatus in chapter IV, orbital and inter-orbital region in chapter V, eye orbital region and face in chapter VI, anomalies of presumably peripheral origin of the extraocular muscles in chapter VII, cornea in chapter VIII, sclera in chapter IX, the anterior chamber in chapter X, uveal membrane in chapter XI, the lens in chapter XII et XIII, and the vitreous body in chapter XIV.

Hydrophthalmus and hereditary forms of glaucoma are included in chapter X. The stationary functional defects of the retina, progressive neuro-retinal abiotrophies and a number of neuro-ophthalmological disorders or syndromes will be dealt with in other Volume in future.

We would have wished to index the subjects and author's at the end of Volume I, but it will be added at the end of Volume II.

Nowadays, the science of genetics makes great progress, and it is very important for biology, medicine and especially for ophthalmology. Therefore we give a hearty welcome to the publication of this very instructive " Genetics and Ophthalmology. Volume I. " We are anxious for the publication of Volume II. We thank you heartily for your kindness that you sent " Genetics and Ophthalmology. Volume I. " to us.

Announcements

The next annual meeting of the Japanese Academy of Ophthalmology will be held July 6-8, 1962, at the Hokkaido University, Sapporo.

General News

April, 1961, Meeting—Japanese Ophthalmological Academy.—The 65th annual meeting

of the Japanese Academy of Ophthalmology held in Tokyo, April 6-8, 1961, Prof. Kakichi Umazume, Prof. of Ophthalmology, Tokyo Medical College, presided and 90 papers on ophthalmology were presented and discussed by about a thousand Japanese ophthalmologists.

The following ophthalmologists from Europe delivered the following themes: Prof. J. François, Belgium, spoke on "Comments on vascularization of the optic pathways". Dr. A. Dubois-Poulsen, Paris, spoke on "New technique for the examination of the peripheral vision".

Papers presented included: A symposium on "the fine structure of the eye; studied with electron microscope", speakers: Prof. H. Fujiyama, Dr. K. Taniguchi and Dr. K. Okuda; "Physiology of accommodation of the eye and its clinical application", Prof. Ryutaro Hagino.

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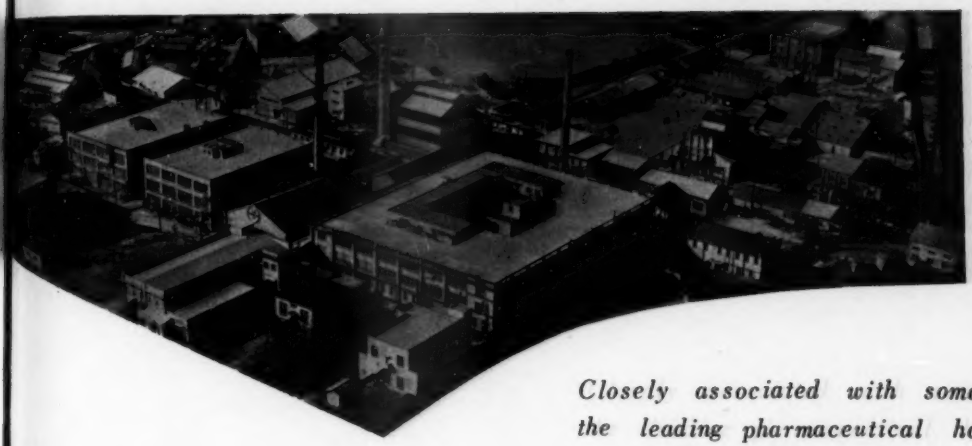

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